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MICRO-BAKING TECHNIQUE, APPLICATIONS AND RESULTS

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(Read at the Annual Meeting, May 1938)

Micro baking is a form of baking procedure on which relatively little work has been reported. Only a few references are to be found in publications of recent years. Werner (1932) did some unpublished work and his terse summation was that "the micro equals the pup divided by four." Geddes and Sibbitt (1933) did work on 25 and 50 g. formulas, described by themselves as limited, and concluded that flours can be satisfactorily differentiated in regard to loaf volume and other characteristics by small baking tests. Geddes and Aitken (1935) did further work in connection with an experimental mill of miniature size and reported a striking comparison in volume and other external characteristics but indicated difficulty in scoring internal factors. Van Scoyk (1937) described a miniature molding device which greatly facilitated handling small doughs and gave much better replication of results. Harris and Sanderson (1938) reported a comparison of 100-g. and 25-g. formulas, finding a significant correlation between the two methods as to volume, stating however that the cut surface of the loaf was too small to score for grain and texture.

Apparatus and Technique

A single micro dough contains 25 g. of flour and the basic A.A.C.C. formula is used, with applications of such supplements as may be required for specific purposes. In fact, reduction of the flour quantity from 100 g. to 25 g. may be considered another supplement to the standard test. The apparatus used is pictured in Figures 1, 2, 3, and 4. Figure 1 shows comparative sizes of the 25-g. and 100-g. pans. Both pans are made of aluminum with the 25-g. pan proportionally scaled down to one-fourth the volume of the larger pan. The ordinary 8-ounce covered jelly glasses shown at the right are used for fermentation jars. Figure 2 shows a side view of the micro-molding assembly with

sheeting rolls on the left and compression drum on the right. Figure 3 shows an oblique top view of the molder. The doughs are given conventional punches by hand and molded by passing through the

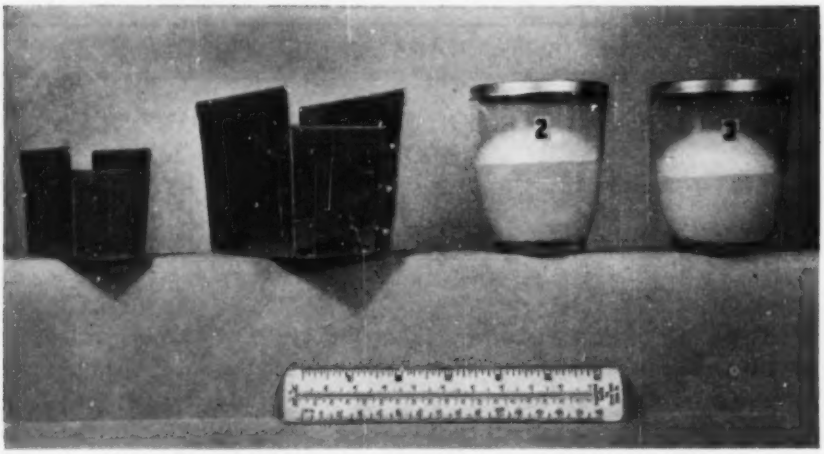

Fig. 1. Pan size comparison and micro fermentation jars.

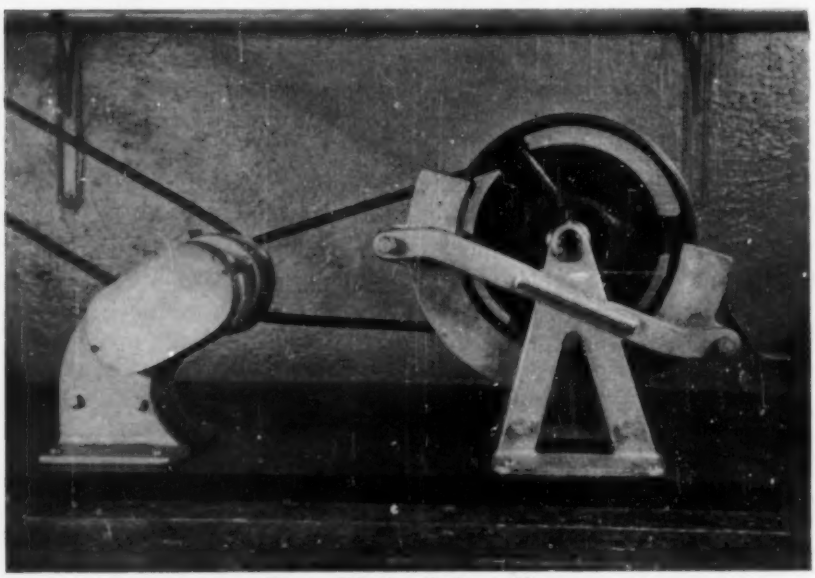

Fig. 2. Side view of micro molder.

sheeting rolls, curling loosely by hand, and then passing through the compression chamber. As to size, this molder is mounted on a base 8 inches by 25 inches and the compression drum is 8 inches in diameter. Standard proof is given the panned dough and baking is carried out

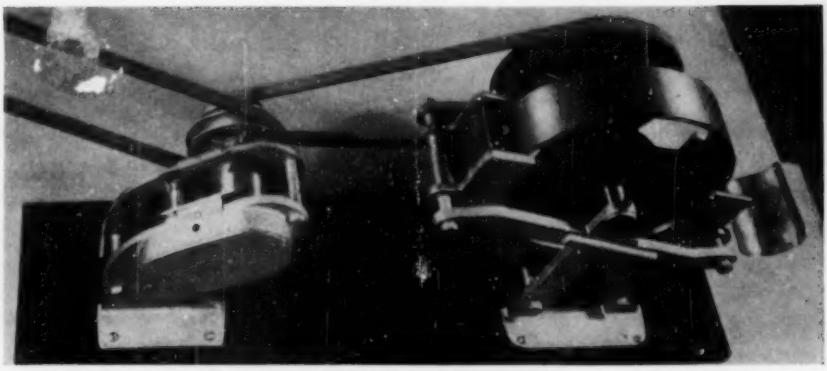

Fig. 3. Top view of micro molder.

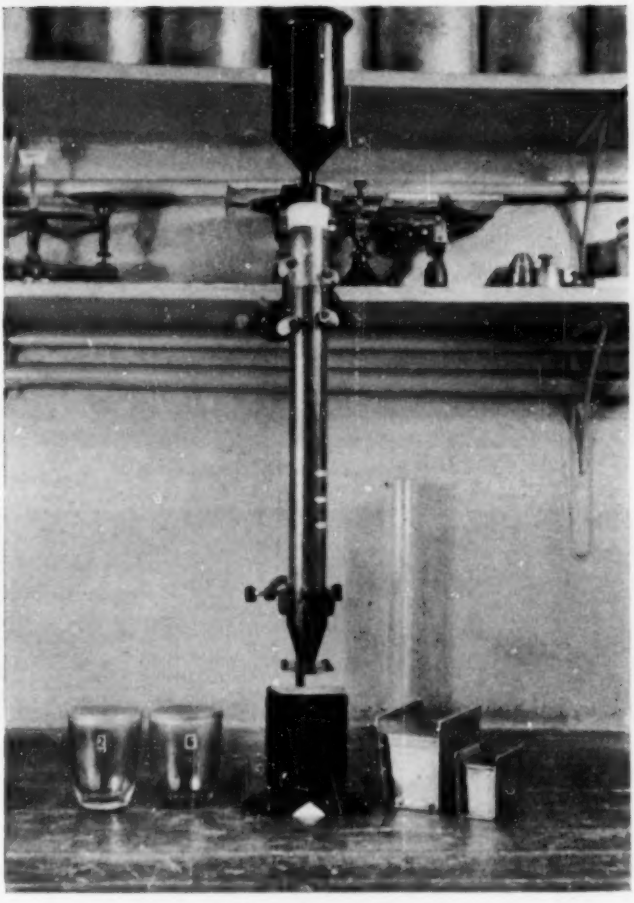

Fig. 4. Miniature loaf volume apparatus.

at 440° F. for 25 minutes. Volumes are measured in a miniature Werner type of apparatus capable of being read to 1 c.c. and shown in Figure 4.

Fig. 5. Fermentation curve using basic 2½% sugar formula.

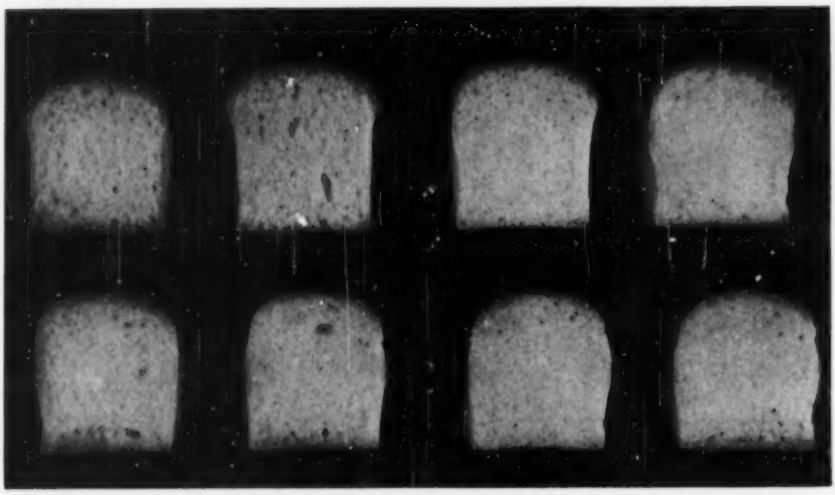

Fig. 6. Cut surfaces of loaves shown in Figure 5.

Fig. 7. Fermentation curve using only flour, water, and yeast.

Applications and Results

1. Because of the small size of necessary equipment micro baking is a convenient means of making fermentation time-volume curves or other series bakes of that nature. For this purpose the entire

amount of flour is mixed at one time and immediately divided into 40-g. aliquots for fermentation. This procedure eliminates a possible

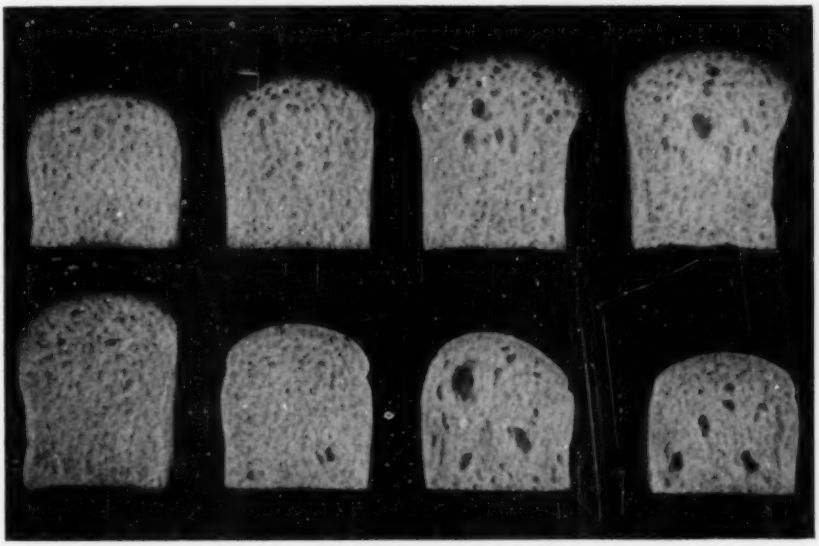

Fig. 8. Cut surfaces of loaves shown in Figure 7.

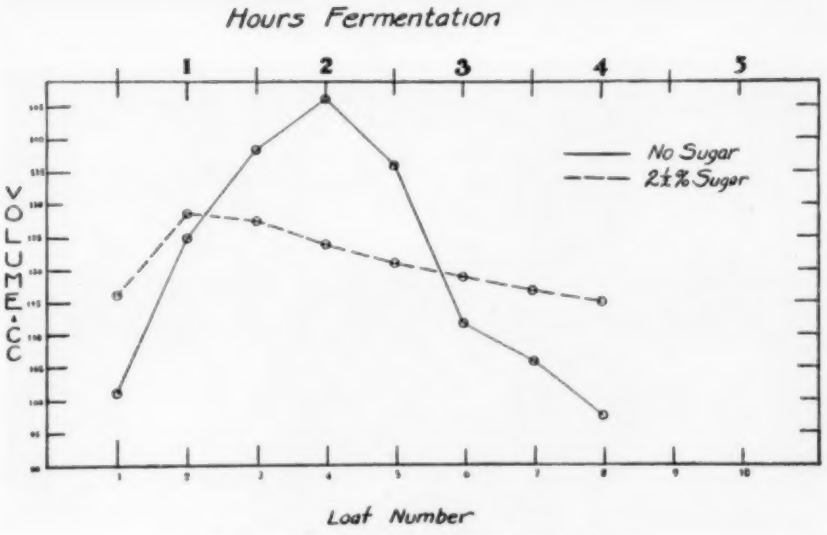

Fig. 9. Graph of loaves shown in Figures 5 and 7.

mixing variable in any one curve. Figure 5 shows a regular basic curve using 2½% sugar. Reading from left to right the times are one-half hour to four hours by half-hour steps. The external forms

of these loaves are as characteristic as loaves of any size would be. Figure 6 shows the internal structure of the same loaves, reading from left to right beginning with the top row. The internal features are

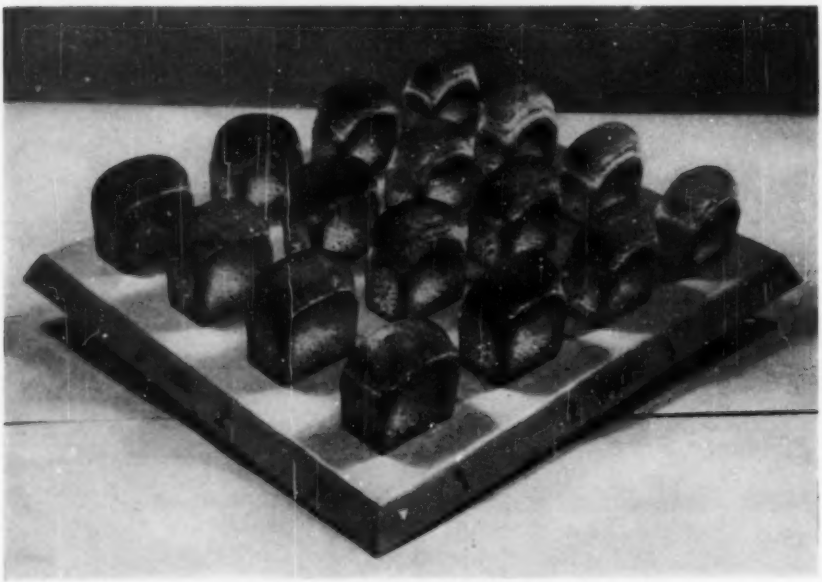

Fig. 10. Checkerboard baking test.

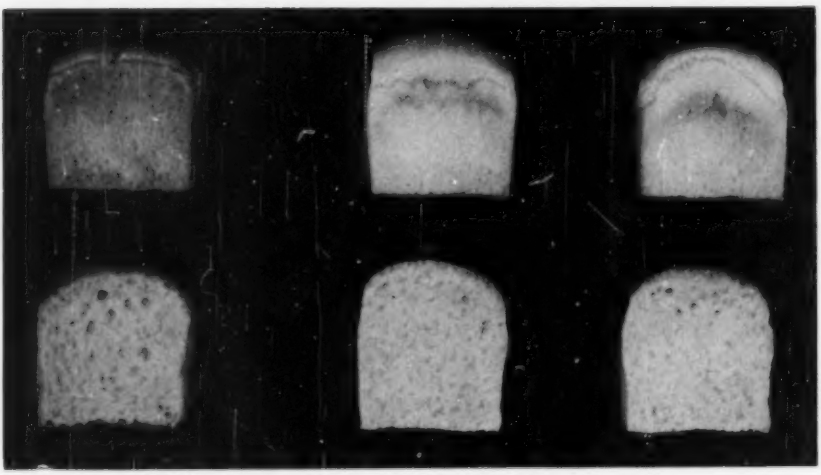

Fig. 11. Representative loaves of checkerboard test (Figure 10).

also as characteristic as they would be in loaves of larger size. Figure 7 shows a sponge development curve made without sugar with fermentation times, left to right, of one-half hour to four hours by half-hour

steps. Figure 8 shows cut surfaces of the same loaves, again reading from left to right beginning with the top row. External and internal features of these small loaves are entirely characteristic. For comparison, Figure 9 shows the two preceding curves in graphical form.

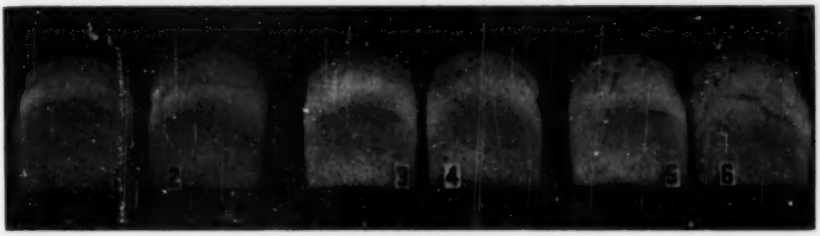

Fig. 12. Routine differential loaves.

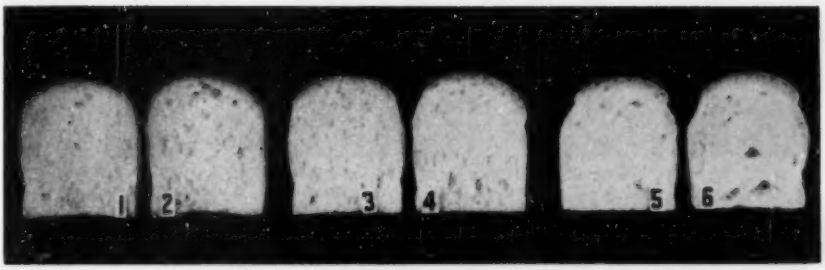

Fig. 13. Cut surfaces of loaves shown in Figure 12.

Fig. 14. Comparison of 25-g. and 100-g. loaves using two types of flour.

Curve baking gives very informative results and the micro method is particularly adapted to this use.

2. The checkerboard, or Latin square, system of baking proposed by Clark (1937) has attracted attention recently. The micro method

is again very convenient for this type of testing because of the size of equipment and precision of handling. Figure 10 shows a composite picture of the results of such a test, with fermentation times varied

Fig. 15. Cut surfaces of loaves shown in Figure 14.

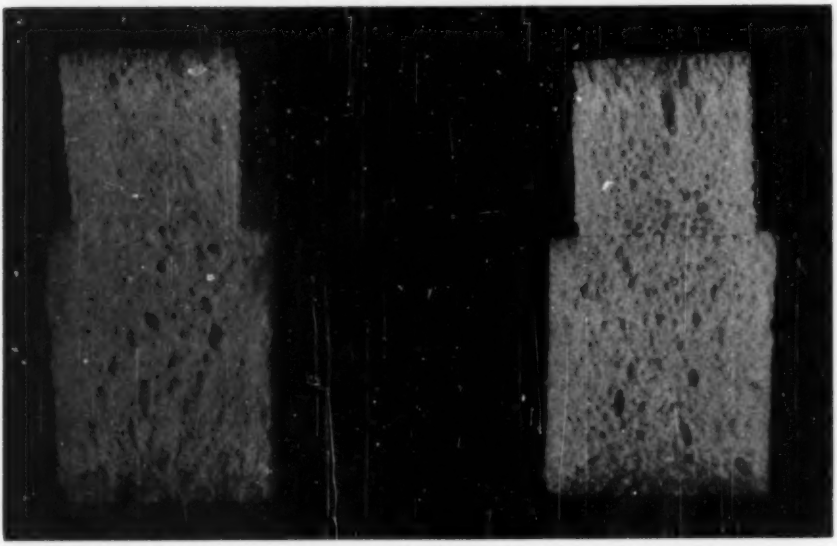

Fig. 16. Enlarged view of sections cut from loaves in Figure 15, eliminating crust.

from one hour to four hours and mixing times from one minute to four minutes. In this case the four loaves representing each mixing time are mixed together and divided into 40-g. aliquots. These 16 loaves are accommodated on a base measuring 15 inches by 17 inches.

Figure 11 shows both external and internal views of the extremes of treatment, together with one of the intermediate loaves. From left to right they are one minute, one hour; 2 minutes, 3 hours; and 4 minutes, 4 hours. The checkerboard system requires a large number

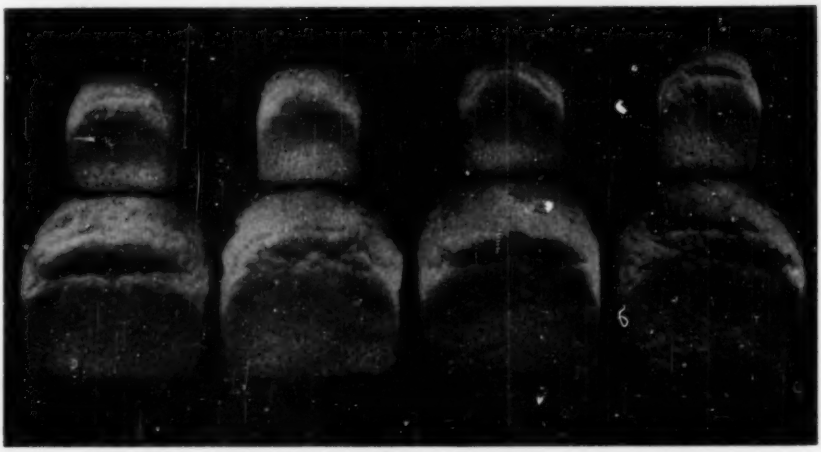

Fig. 17. Comparison of 25-g. and 100-g. loaves in two other types of flours.

Fig. 18. Internal view of loaves shown in Figure 17.

of loaves and can be nicely handled by this method without excessive-sized fermentation and oven equipment.

3. Micro baking is equally applicable to routine differential baking tests. For this purpose 100 g. is mixed and unused portions discarded. Figure 12 shows external views of three flours of similar type but of decreasing strength from left to right. The even-numbered loaves

are the bromate supplements, all having 3½ hours of fermentation time. Figure 13 shows internal features of the same loaves. Loaves 1 and 2 have a rather rugged type of grain and 3 and 4 the elongated type, while 5 and 6 have begun to show a little distress, particularly in the plus loaf. Results pictured in both external and internal views can be interpreted as easily as results obtained with larger-sized loaves.

Fig. 19. Further comparison of types in 25-g. and 100-g. formulas.

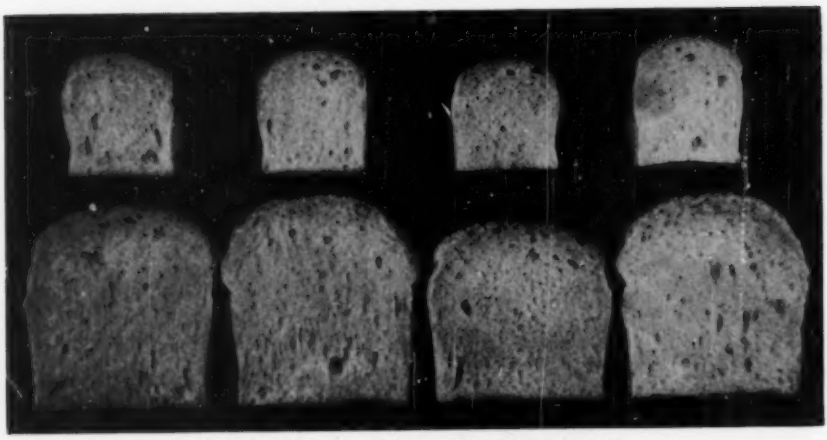

Fig. 20. Cut surfaces of loaves shown in Figure 19.

Figure 14 shows a comparison of two types of flours in 25-g. and 100-g. formulas. The right-hand loaf of each pair is the bromate supplement. The 100-g. loaves were molded by the standard hand procedure; otherwise baking conditions were the same. Figure 15 shows cut surfaces of the same loaves. It is possible that some difficulty encountered in judging small loaves for grain and texture may have been due to the different crust-crumb ratio. Figure 16 shows

sections cut out to eliminate viewing the crust. The smaller top portion of each pair is from the micro loaf. Variations in grain and texture are within the limits shown by Moen (1935).

Figures 17 and 18 show further external and internal comparisons of different types and Figures 19 and 20 a third comparison. In the comparative pictures all of the flours were of different types or grades. The right-hand set of loaves in Figure 20, for example, are typical of an unbleached flour low in diastase.

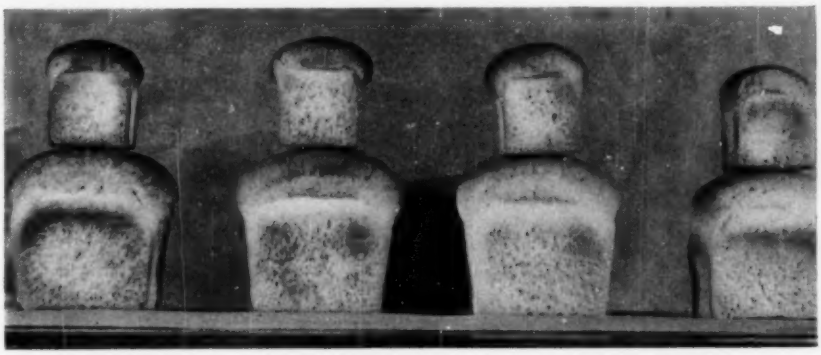

Fig. 21. Comparative results with both sizes of loaves machine molded.

Figure 21 is the only available photograph of a comparison in which both sizes were machine handled. The larger loaves were molded on a commercial molder adapted to 100-g. size. Considerable collaborative work of this kind was done but not recorded photographically and the closing of the collaborating laboratory prevented further work. In this photograph the two sets of loaves show almost identical characteristics.

Summary

The micro baking procedure is described and the apparatus and typical results and comparisons are shown. The results of the three types of testing described are as informative as results obtained with larger loaves. Quantity baking without excessive-sized equipment, precision, elimination of variables in some cases, and greater ease of replication of results are some of the advantages of the 25-g. procedure. When mechanical equipment is used there is no difficulty in obtaining satisfactory differentiation of both external and internal characteristics.

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RECORDING-MIXER MEASUREMENTS OF CHANGES IN GLUTEN QUALITY DURING FERMENTATION [1]

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(Read at the Annual Meeting, May 1938)

Recently a paper (Malloch, 1938a) was published describing a recording mixer for small samples. Evidence was presented showing that the breaking time, that is, the time of occurrence of a characteristic break in the downward course of the curve, is probably related to gluten quality. The results of further investigation of this relation are now given.

Plan of Experiment

Since no satisfactory independent measure of gluten quality of proved validity exists, recourse must be had to its inference from the results of baking tests. However, as these vary considerably with the baking procedure used, it is evident that the relation between loaf volume and the quality of the protein *as it exists in the original flour* must be indirect. The colloidal characteristics of the flour protein will be modified by the addition of other dough ingredients and still further during fermentation. It is useless, therefore, to expect a close relation between the results of baking tests and the results obtained with any kind of mechanical dough-testing device working on flour-and-water doughs, unless (1) the modification of the gluten by the other ingredients and by fermentation is comparable for all flours tested, or (2) the differences in the gluten of the original flours are so great that the variations in modification are masked. The first condition is unlikely to be realized in practice and when the second applies no information on the utility of the machine to distinguish small differences can be obtained. This difficulty can be overcome by making the measurements on fermented doughs at a time corresponding to

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the end of the proofing period, and this was the procedure adopted in this investigation.

Since, with any given oven conditions, the loaf volume will depend on the protein content of the flour, the colloidal properties of the dough at the time of baking, and the gas production, it is possible, by eliminating the effect of variability in protein content and gas production, to assess the colloidal properties by obtaining the loaf volume. In this experiment the effect of differences in the protein content of the flours used was eliminated by studying only the changes in loaf volume and breaking time at varying fermentation times *within* each flour, and the effect of variation in gas production was allowed for by appropriate statistical calculation.

Twenty flours of varying quality milled from United States wheat in a commercial mill were used. Table I gives a description of the

TABLE I
EXPERIMENTAL MATERIAL

Origin	Class of wheat	Flour protein	Loaf volume
		%	*c.c.*
Elevator lot	Winter	10.0	462
Elevator lot	Winter	10.7	502
Elevator lot	Winter	11.7	528
Iowa	Winter	11.3	578
Iowa	Winter	11.2	473
South Dakota	Spring	13.7	593
South Dakota	Mixed spring	14.8	582
South Dakota	White	12.4	613
South Dakota	Spring	14.2	554
Kansas	Winter	12.1	608
Kansas	Winter	11.1	543
South Dakota	Spring	14.5	671
Elevator lot	Spring	15.2	684
Minnesota	Spring	12.6	607
North Dakota	Spring	12.8	615
Minnesota	Spring	12.7	671
Minnesota	Spring	13.8	696
North Dakota	Spring	13.0	677
North Dakota	Spring	13.1	625
Elevator lot	Spring	14.0	688

wheats with the protein content of the flour and the loaf volume obtained, using the malt-phosphate-bromate formula and the usual A. A. C. C. procedure and fermentation time.

Each flour was baked by the malt-phosphate-bromate formula at five fermentation times varying from 1 to 5 hours. Recording-mixer tests were made at times corresponding to the end of the proofing periods on successive portions of a dough mixed by the same formula, using the same absorption and fermented under the same conditions. The gas production during the proofing periods was determined by the

Blish method (Blish, Sandstedt, and Astleford, 1932, and Sandstedt and Blish, 1934) by means of an apparatus recently described (Malloch, 1938b).

Results

The results of the three determinations are summarized in Figure 1, which shows the average values for the 20 flours at each fermentation time. The rough relationship, that loaf volume increases with increasing gas production and with decreasing breaking time, is apparent from this graph.

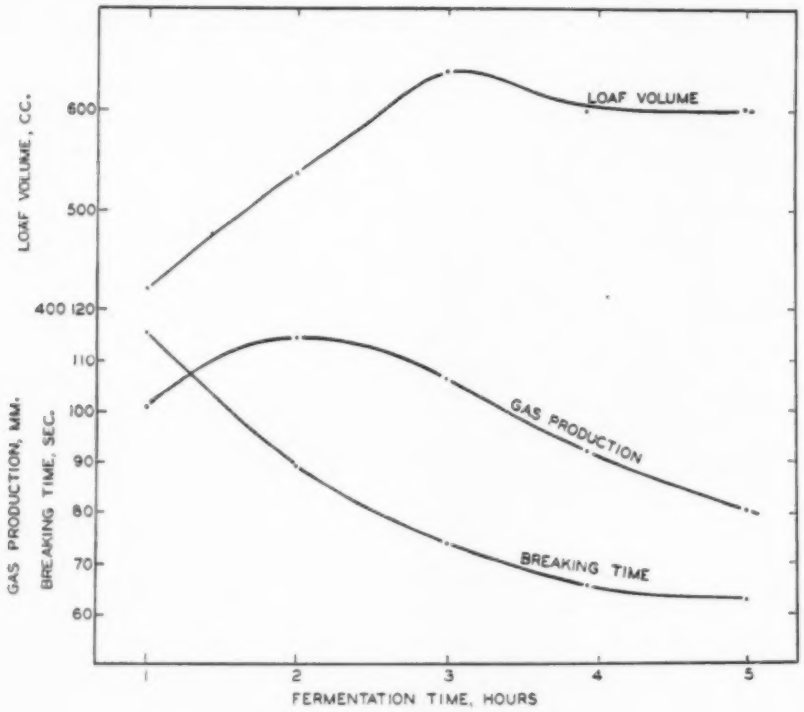

Fig. 1. Average values for 20 flours.

In Figure 2 a series of curves obtained with a single typical flour are shown. These show the characteristic differences between a curve for a flour-and-water dough and one for a complete dough and also the subsequent changes due to fermentation. The corresponding changes in breaking time for the average of the entire series are shown in Figure 3 together with the effect of fermentation on the breaking time of two individual flours. These curves confirm the necessity of comparing the loaf volumes with the breaking times obtained under comparable conditions.

Little information regarding the nature of the relations involved can be obtained by a simple examination of the data and recourse must be had to statistical analysis.

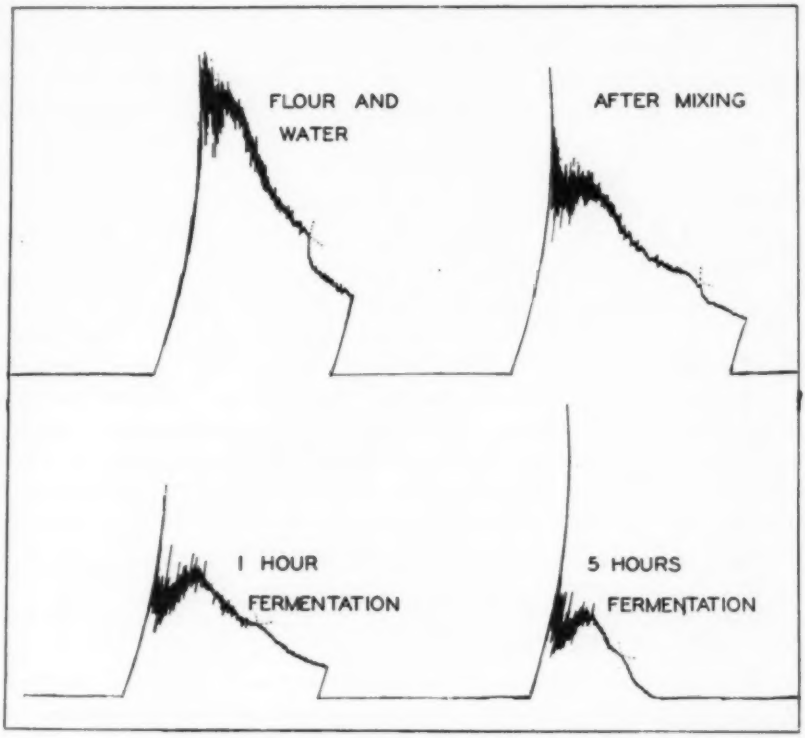

Fig. 2. Curves obtained with a single characteristic flour showing changes due to mixing of usual dough ingredients and fermentation.

Statistical Analysis

The variance and covariance within flours were computed and used as the basis for the calculation of the partial regression and multiple correlation coefficients relating the changes in gas production for different proofing periods, and breaking time at the end of different proofing periods, with the variation in loaf volume due to difference in fermentation time. The first calculation was made on the assumption that the relations between gas production and loaf volume and between breaking time and loaf volume were both linear. Then a curved relation was assumed for gas production only and a quadratic term inserted in the gas-production portion of the equation. Finally it was assumed that both relations are curved and a quadratic term was

inserted for breaking time also. The results of these calculations are given in Table II.

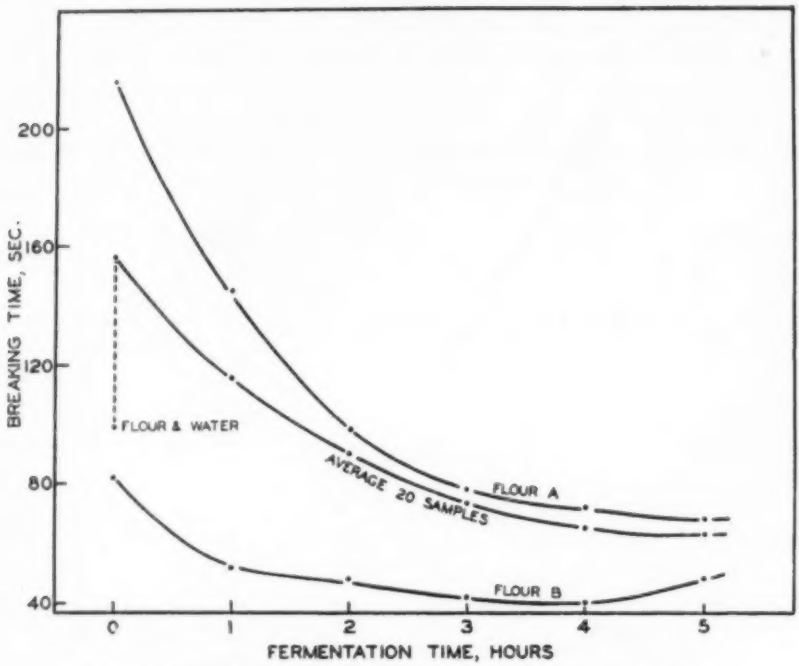

Fig. 3. Average values for the 20 flours and values for two individual flours.

TABLE II

RELATION BETWEEN CHANGES IN GAS PRODUCTION AND BREAKING TIME, AND CHANGES IN LOAF VOLUME WITHIN FLOURS

Partial regression coefficients	Multiple correlation coefficient, R
$+ 2.67P - 3.26T$	.84
$+ 33.32P - 0.158P^2 - 3.27T$	.90
$+ 16.04P - 0.060P^2 - 12.50T + 0.036T^2$	.97

P = gas production during proofing period (mm.)
T = breaking time at end of proofing period (sec.)

The proportion of the variance in loaf volume accounted for increased progressively with the insertion of each quadratic term. The significance of these was tested by calculating the residual variance in each case and comparing the variance accounted for by the regression terms with the mean square residual. The F values were 127.1 and 165.7, respectively, while the value of F at the 1% point is 7.0. The curvature of both relations is therefore highly significant.

With the double quadratic equation the residual variation of loaf volume as measured by the standard deviation is only 23.32 c.c. This may be compared with the experimental error of the determination of loaf volume given by the standard error of the mean of replicate determinations (7.82 c.c.). It is possible that better fits could be obtained by the use of higher-order equations, but the residual variance is already so low that the very considerable labor involved in fitting cubic terms does not seem to be justified.

Discussion

The statistical analysis shows that there is a very close relation between the variation in loaf volume within a single flour at different fermentation times and the changes in gas production and breaking time of similar doughs measured at comparable times. The residual variation in loaf volume unaccounted for by the change in the other variables is only 23 c.c., and even this small error can be partly accounted for by experimental errors in the three determinations involved. Thus it is established that in the series of samples studied the effect of varying frementation time on loaf volume can be attributed to the changes in gas production during proofing at different periods and to the variation in some factor which is measured by the breaking time determined at the end of the proofing period. The relations involved are both markedly non-linear. As the gas production increases a smaller proportion of the total gas is retained and the curve relating gas production and loaf volume flattens out. Similarly as the breaking time becomes shorter the loaf volume increases and the change is more pronounced with the shorter breaking times.

The samples covered a wide range of flour quality (Table I) and this with the different fermentation times gave a wide variety of baking conditions. The extreme range of loaf volume was from 392 c.c. to 755 c.c. Gas production during the proofing period varied from 71 mm. to 121 mm. and the breaking time at the end of the proofing period from 40 sec. to 210 sec. Since five fermentation times were used with each of the 20 flours, 80 independent estimates of the changes due to fermentation were available as a basis for the conclusions. The very close relation discovered over the entire series shows that each individual flour must have behaved in a similar manner. In view of the wide variety of baking conditions and the similar behavior of all the flours, there is a strong presumption that the relation found in this series will have general application.

The variation in loaf volume within a single flour can only be caused by changes in the gas production and colloidal properties and it has been shown to be a function of changes in gas production and breaking

time. Therefore, the breaking time must be related to the colloidal characteristics of the dough. These colloidal characteristics are determined mainly, if not solely, by the condition of the gluten, generally called "gluten quality." Hence, with respect to the properties affecting loaf volume, the changes in gluten quality during fermentation must be indicated by changes in the breaking time with a high degree of accuracy. It is not suggested that the changes in the properties measured by the breaking time are the only ones taking place during the fermentation of dough. Indeed the character of the recording-mixer curves for various fermentation times is good evidence to the contrary. However, if any of the other properties influence loaf volume, then the changes in them must be closely correlated with the changes in the properties affecting breaking time, which, in actual practice, gives a measure of the combined effect.

Since the changes in gluten quality are measured accurately by changes in the breaking time, the utility of the breaking time of flour-and-water doughs as a measure of gluten quality of the original flour is strongly indicated. However, the ability of the breaking time to distinguish differences between the gluten qualities of different flours must be established before this conclusion in logic can be established in fact. The present series is too short for investigations of this kind, as only 19 effective comparisons between flours are available as contrasted with the 80 on which the present conclusions are based.

The pronounced effect of gas production during the proofing period on loaf volume, which is established in this investigation, gives definite experimental confirmation to the importance which has been generally attached to the gassing power during this phase of baking. It is, of course, particularly important in test baking. In the baking test using the regular A. A. C. C. procedure, which was conducted as part of the experiment, the gas production during the proofing period was 83 mm. for the lowest-gassing flour and 104 mm. for the highest, in spite of the use of high diastatic malt and phosphate in the formula. This difference would be sufficient to account for a difference in loaf volume of approximately 100 c.c. The interpretation of the results of baking tests in terms of the strength o the flour may be seriously in error if differences in gas production of this magnitude are not taken into account. However, in any attempt to equalize the gas production, the continuous modification of gluten quality during fermentation should receive due consideration.

Summary

The utility of a new recording mixer using small samples for following with a high degree of accuracy the changes in gluten quality during fermentation is demonstrated.

Proof of the importance of controlling gas production during the proofing period in test baking is given.

Acknowledgments

I am indebted to Dr. C. H. Bailey for supplying the samples used in this investigation and to Dr. J. W. Hopkins of these laboratories for his advice on the statistical analysis of the data.

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THERMOSTATIC DOUGH-TEMPERATURE CONTROL FOR HOBART-SWANSON MIXER

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(Read at the Annual Meeting, May 1938)

The factors which influence the mixing of a dough seem to be somewhat complex and of a varied nature. Skovholt and Bailey (1932) have shown that the height of the farinograph curve varied markedly with temperature. Bohn and Bailey (1936) report extensive studies as to the effect of mixing on the physical properties of the dough. Talbott and Weaver (1933) devised an apparatus for the convenient and accurate delivery of solutions used in the experimental baking test. Other cereal chemists have noted the importance of temperature control on dough behavior, and have spent considerable time and study on these factors.

Procedure

Having access to a thermostat used as a temperature-control unit with the Brabender farinograph, we have limited our investigation to include only the possibility of controlling the temperature of the dough during the mixing stage. The thermostat used was found to be very efficient in water of the desired temperature circulating through a water bath fastened to the mixing bowl of a Hobart-Swanson mixer. Pre-

liminary tests showed that the aluminum mixing bowl is an excellent conductor of heat.

The temperature factors, which may partly or wholly be controlled in the laboratory, vary considerably from day to day, and must be considered in the light of the ultimate results obtained. The temperature of the dough from the mixer is an important factor to consider either in the laboratory or bakery, and is controlled as accurately as the equipment used will permit.

The temperatures considered in this report as affecting the mixing stage were those of the room, flour, water, yeast, salt, sugar, bromate, and any other ingredient added in either a dry or a liquid state. Solutions of the ingredients are made to conform to those specified in the A. A. C. C. experimental baking test, which greatly facilitate temperature readings.

It was deemed advisable to take the temperature of the solutions and ingredients just prior to mixing, and to repeat this procedure at

TABLE I

TEMPERATURE INCREASE (F) OF SOLUTIONS IN MIXING BOWL

Temperature of room, water, and solutions used in A. A. C. C. exp. baking test [1]	Thermostat temp.	Temperature of solutions in mixer at time intervals (minutes)			
		1½	3	4½	6
Av. ° *F.*	° *F.*	° *F.*	° *F.*	° *F.*	° *F.*
77.2	84.2	82.0	82.5	82.5	83.0
77.2	86.0	83.5	83.5	84.0	84.5
77.9	87.8	86.0	86.5	86.5	86.5
79.0	89.6	87.0	88.0	88.0	88.0
79.0	91.4	88.0	88.0	88.5	89.0
79.0	93.2	89.0	90.0	90.0	90.5

[1] Water based on 65% absorption.

TABLE II

DOUGH TEMPERATURES UNDER THERMOSTAT CONTROL IN A HOBART-SWANSON MIXER

Flour No.[1]	Water	Mixing time of dough	Temperature of room, water, and solutions added in A. A. C. C. baking test	Thermostat temp.	Dough temp. from mixer
	%	*Min.*	*Av.* ° *F.*	° *F.*	° *F.*
1	60	1½	79.0	86.0	85.0–85.0
2	64	2	75.0	89.6	85.0–85.0
3	65	2	79.0	87.8	86.0–87.0
4	66	2½	78.0	87.8	86.0–86.0
5	65	2½	78.0	87.8	85.0–85.0
6	65	2½	77.0	87.8	85.0–85.0
7	65	2½	79.0	87.8	86.0–86.0
8	68	3	78.0	87.8	87.0–86.0

[1] No. 1—weak family flour. Nos. 2, 3, 4, 5—hard winter wheat flours. Nos. 6, 7—spring wheat flours. No. 8—spring clear flour.

definite time intervals. Any appreciable change in the temperature of the solutions added would of course necessitate a thermostat correction, all other factors being constant. In order to obtain if possible a more

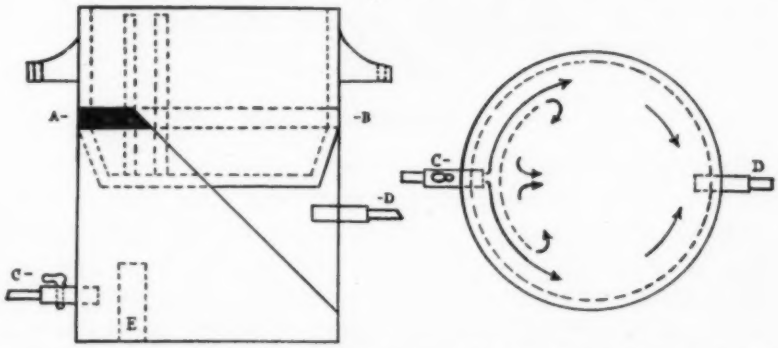

Fig. 1. Diagram showing water bath fastened to bowl of Hobart-Swanson mixer. *A*, steel band supporting bath to mixing bowl. *B*, rubber facing to prevent leakage. *C*, inlet. *D*, outlet. *E*, curved fin.

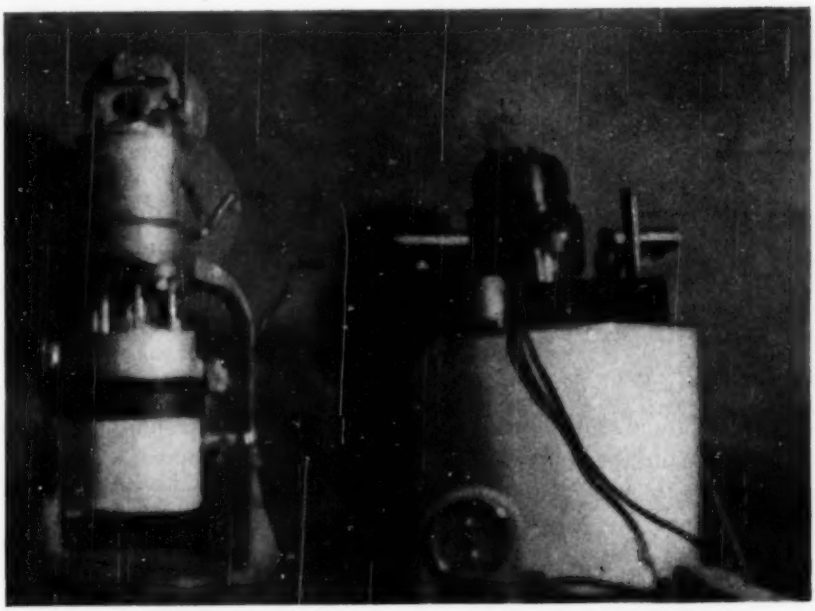

Fig. 2. View showing Hobart-Swanson mixer and thermostatic temperature control.

comprehensive picture as to the degree in which the thermostat indicates temperature changes of the solutions in the mixer, a series of tests were made at intervals of one and one-half minutes (Table I). The designated interval of time approximated somewhat closely the laboratory mixing schedule.

The flours which are reported in Table II were chosen at random from data accumulated over a considerable period of time, and under temperature variations experienced in the laboratory on that particular day. Both hard-winter and spring wheat flours are listed, using a variable mixing time and absorption (500 units) approximating that obtained on the Brabender farinograph.

Discussion

From the data shown and accumulated over several months of continuous operation, it would seem that thermostat control has some value in mixing. The results do not seem to indicate that any appreciable heat is generated in mixing, which is probably due to the even temperature of the surfaces with which the dough comes in contact. There seems to be a certain loss of heat due to radiation in the mixer and from the tubing leading to the thermostat, which accounts for the lower temperature reading of the solutions in the mixer.

The order in which the solutions are added to the mixing bowl seems to influence the temperature of the resultant dough. The distilled water, being the chief ingredient in terms of volume and likewise with few exceptions the coldest, was placed in the mixer first. This was followed by adding the salt and sugar, bromate, and yeast solutions. A thermostat temperature range of 30° to 33° C. has been found applicable to existing temperature variations from day to day. During the extremely warm weather of the summer months, the thermostat has been operated successfully to control the dough temperature. The relatively short time that it takes to increase or decrease the temperature of the thermostat makes it a very useful piece of equipment in the laboratory.

Summary

A thermostat control may be used effectively in controlling the dough temperature in the mixer. The accuracy of the control will permit results varying not more than ± 1° F. from the dough temperature desired.

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REPORT OF THE 1937–38 COMMITTEE ON STANDARDIZATION OF LABORATORY BAKING [1]

C. F. DAVIS, *Chairman*

Western Star Mill Co., Salina, Kansas

(Read at the Annual Meeting, May 1938)

During the past year the committee has endeavored to carry on work along lines recommended by the 1936–37 committee. It appears that some definite progress has been made but the work of the Committee on Standardization of Laboratory Baking is not to be measured in terms of a year's work.

Development work on all test baking equipment is in progress. The development of sheeting and molding equipment has been encouraged to the extent that two papers are offered for this program relating to the possible value of such equipment. Manufacturers have been encouraged to the extent that we have exhibited at this convention some sheeting and molding machines which are available at moderate cost and which are definite improvements over hand operations. In order to cooperate with manufacturers and facilitate immediate further development of molding equipment this committee has unaminously agreed to establish a 10 cm. bottom pan length with the idea of offering next year for the approval of the association an arbitrary pan built around this dimension and suitable as an optimum pan size for the loaf volume range of from 550 to 750 c.c.

Two contributions on the subject of sponge dough laboratory baking tests are offered on this program and further contributions are in the process of development.

This committee would recommend that further work be done as recommended by the 1936–37 committee.

[1] General report.

HAND PUNCHING AND HAND MOLDING VS. MACHINE PUNCHING AND MACHINE MOLDING [1]

W. L. HEALD

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(Read at the Annual Meeting, May 1938)

For the past few years the Baking Committee has recognized the problem of standardized equipment for the successful operation of the baking test if it is to continue as the official baking test of the American Association of Cereal Chemists.

Previous Investigations

G. Moen (1929) published results of the Baking Committee's work for that year in which he found between two operators a difference of 5.4% in loaf volume, while the grain and texture also were manifestly different. In the interest of securing more uniformity through mechanization, the Thompson Machine Company developed a one-man molder, type "I." Fifield and Weaver (1930), of the U. S. Department of Agriculture, secured the use of one of these machines and reported that whereas Moen had experienced a 5.4% difference in loaf volume with a molding machine and different operators, a variation of 1.9% was experienced in their work. Thus it would seem that a step had been made in the right direction.

Merritt and Blish (1931) carried out additional work with a Thompson molder. Their conclusions were that while the machine gave greater uniformity than hand molding, they still encountered some variations which they were unable to overcome in any adjustment made on the machine. The same year Geddes and Goulden reported an extensive study of the variability as influenced by mechanical molding involving more than 4,000 loaves in which the personal factors of punching and molding were studied. Result of this work was as follows: "Hand molding does not appear to be a factor of major importance in causing variability between replicate bakes. Since punching and molding personality both contribute to the variability between bakers, the introduction of mechanical molding machines may be expected to reduce but not eliminate the large difference in mean loaf volume which different operators working in the same or different laboratories secure in replicate bakings of the same flour."

[1] Sub-committee report, 1937–38 Committee on Standardization of Laboratory Baking.

Merritt, Blish, and Sandstedt (1932), on further work of the A. A. C. C. fellowship with machine molding, found that the personal factor in dough punching was perhaps the cause of the greatest variation. In view of this fact, a pair of sheeting rolls were devised called the "S" rolls. These rolls operated by hand and, as reported in the work, tended to reduce variation. Geddes and Sibbit (1933) also reported that the use of the "S" rolls very definitely tends to reduce variability between operators.

Freilich (1933) also reported that sheeter molding has a tendency to produce a smaller variation than hand molding and that the sheeter molder gave no better concordance among the mean volumes obtained with different flours than hand molding. A further report of Freilich (1935) with a motor-driven sheeter was as follows: "The sheeter is decidedly to be preferred over hand molding if one wishes to obtain better results when comparing different samples on a given day in his own laboratory. Molding by hand is to be preferred over the sheeter when better agreement between different laboratories is desired."

Discussion of Sheeter and Molder

The writer had constructed a pair of "S" rolls in 1934 as outlined in Cereal Chemistry, **9**: 195, except that they were motor driven. Some experimentation as to the speed of the rolls was carried out and a speed of 150 r.p.m. was found to be satisfactory.

Having definitely established a procedure, we have used the method for the past four years in all our punching and sheeting prior to hand molding. VanScoyk (1937) described a molder devised to mold micro loaves very satisfactorily. The writer, believing that a molder designed to handle the A. A. C. C. baking procedure would be very desirable, decided to construct one for his own use. Since we had the sheeting rolls in operation, it was decided to build the apparatus as outlined by VanScoyk, that is, the molder itself separate from the sheeter. It is not a question of speed in the laboratory molding operation, but one of precision, and it was thought that perhaps some of the variation might be eliminated by curling or rolling the doughs by hand between the operations with the sheeter and the molder.

Since we had no data as to the diameter or the width of the drum which would give satisfactory results we had to carry out a series of bakes using different widths of drums and compression plates. At first we decided on a drum 16 inches in diameter and 3½ inches wide. With an adjustable compression plate, we tried a great many settings but were never able to get a satisfactorily molded loaf that fit the A. A. C. C. tall-form pan. We did, however, get a loaf molded satisfactorily for the low-form pan.

Since the sides of the compression plate were adjustable we decided to try a drum 16 inches in diameter and 5 inches wide on the face. This, of course, necessitated making a new compression plate. This drum and plate we have found to give excellent results with the following compression plate settings.

The entrance end is set 1½ inches from the drum and at the discharge end 1 inch from the drum. Slots are cut in the side plates so that adjustments can be easily made. The speed of the drum is 30 r.p.m. Both faces of the drum and the compression plate are covered with cotton belting.

Operation of Molder

The procedure in connection with the molder is as follows: A. A. C. C. formula and fermentation time. At punch time the dough is removed from the fermentation bowls as carefully as possible. The wet edges are folded together, and the ball of dough is elongated slightly and placed through the sheeter rolls, lightly rolled up and again placed in the fermentation bowl.

The second punch is the same as the first. At pan time the dough is again removed and passed through the sheeting rolls. This time the dough is laid on a board covered with cotton belting and again rolled the same as in the punching procedure. The lightly rolled dough is placed in the molder with the loose end or flap as far away from the revolving drum as possible so that the seal will not have a pocket of occluded air and show large holes in the baked loaf. When the molded dough comes from the molder it is slightly longer than the pan but the dough draws up slightly during the placing in the pan, which we find just fills the pan nicely. If it should have a tendency to hang on the ends, a flat spatula places it in position very easily. In fact we have found it a good practice to use a spatula on all the loaves. Since doing this we have practically no loaves with low or bulging ends.

Discussion and Summary of Results

To see if a more consistent baking procedure could not be established by use of the sheeting rolls and the molder, a series of daily bakes were carried out in duplicate extending over a period of a month. Each day a series of eight loaves were baked as follows and illustrated in Table I:

A—machine punched and machine molded
B—machine punched and hand molded
C—hand punched and machine molded
D—hand punched and hand molded

TABLE I

AVERAGE LOAF VOLUMES IN CUBIC CENTIMETERS OF DUPLICATE BAKES

No.	Machine punched, machine molded A	Machine punched, hand molded B	Hand punched, machine molded C	Hand punched, hand molded D
1	551	512	515	478
2	527	521	515	490
3	539	506	521	509
4	536	512	533	524
5	521	509	512	492
6	527	527	536	512
7	542	521	498	490
8	527	506	507	521
9	518	493	504	488
10	515	495	495	500
11	527	521	506	521
12	539	506	515	496
13	525	500	509	495
14	527	490	536	506
15	542	542	551	545
16	533	509	553	524
17	512	485	521	488
18	521	515	504	485
19	527	515	521	518
20	542	524	506	512
21	530	509	515	506
22	548	509	539	506
23	527	509	509	485
24	521	506	495	492
25	551	530	527	524
Mean volume value	531.0	510.84	517.88	504.36
S. E. of duplicate determination	9.85	9.28	11.88	11.60
Standard deviation	10.89	13.00	16.11	16.54
Range	39	57	58	67

We find from Table I that the mean volumes of the machine-punched and machine-molded loaves were consistently larger, and also that the standard deviation as well as the range is smallest for the machine-punched and machine-molded loaves.

While loaf volume as reported in cubic centimeters was of most importance in this series of tests, it was also noted that the more uniform grain and texture were to be found in the machine-punched and machine-molded loaves. In other words, the machine-punched and machine-molded loaves had fewer holes and practically no large holes in any of the bakes made, while the hand-punched and the hand-molded loaves tended to have slightly less uniform grain and texture.

We find also that the standard error between duplicates is slightly lower in the machine-punched and hand-molded loaves, the "B" series. The reason for this is not particularly evident. The range of loaf volume between duplicates is less in the case of the machine-

punched and machine-molded loaves and greatest in the hand-punched and hand-molded loaves. It is interesting to note that the hand-punched doughs, namely "C" and "D," are considerably higher in standard deviation than "A" or "B." This tends to substantiate data already reported to the effect that as a personal factor the punching is more of a deciding factor than the molding.

It was impossible for us to carry out a series with an inexperienced operator, but it is believed, as has been reported by other workers, that the machine-punched and machine-molded loaves would, with unskilled operators, materially reduce the variation to be expected.

Results as here illustrated are not as much in agreement as we had hoped for. We feel, however, that machine punching and machine molding are to be desired over hand punching and hand molding.

Statistical Discussion

Using four handling methods, loaves were baked in duplicate on each of 25 successive days, the same previously aged flour being used throughout. An analysis of variance was run on the data, and the interaction thus obtained was found to be significant as compared with the duplicate error, and consequently was used as a basis of comparison for the variances attributable to days and handling methods. In addition, the standard errors for duplicate determinations for each handling method were calculated. These were 9.85 c.c., 9.28 c.c., 11.88 c.c., and 11.60 c.c., for methods A, B, C, and D respectively (Table I).

The day-to-day fluctuations were reflected in the standard deviations of the means of duplicates for each of the four handling methods. The differences between days have already been demonstrated to be significant, but it was also desired to know whether or not the variabilities within each method were also significantly different. This was done. The variability in the A method was significantly less than in either C or D, although not less than in B. This would further indicate the greater effect of the punching as a personal factor than the molding.

Considering all the statistical results it would seem that method A is the most desirable. It gives the largest loaf volume, has a small duplicate error, and may be more closely replicated in different bakes as shown by the lower daily variability.

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COOPERATIVE TEST OF A PUNCHING AND MOULDING MACHINE[1]

J. G. MALLOCH

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(Read at the Annual Meeting, May 1938)

In 1935 a paper (Malloch and Hopkins) was published describing briefly a machine for punching and moulding 100-g. doughs and giving the results of preliminary investigation of its utility. Since that time a new model of the machine has been built with refinements in the constructional details. This model was tested cooperatively in four Canadian laboratories.

Description of Experiments

Experiment 1. Duplicate samples of fifteen commercially-milled flours, varying in protein content from 10.4% to 15.1% were baked on eight days in each of four laboratories. The order of baking was randomized for each day. One of each pair of doughs was punched and moulded by hand and the other by machine.

Experiment 2. Thirty loaves of a single flour were baked on each of four days in each laboratory and alternate doughs were punched and moulded by machine and by hand.

Every effort was made to have uniform conditions in the cooperating laboratories. The same Hobart-Swanson mixer was used throughout, with a mixing time of 1½ minutes. The moisture contents of the flours and the correct absorptions were determined by one of the laboratories. The flour was shipped in moisture-proof containers and

[1] Published as Paper No. 140 of the Associate Committee on Grain Research, National Research Council of Canada and Dominion Department of Agriculture.
Sub-committee report, 1937–38 Committee on Standardization of Laboratory Baking.

each laboratory used the specified absorptions. The malt-phosphate-bromate formula was used with the A.A.C.C. baking procedure and all laboratories used low-sided pans. Three extra loaves were included at the beginning and end of each day's baking and discarded. All the laboratories did at least two days of trial baking using the machine before the experiments were started.

Results and Discussion

The results of an analysis of variance of the loaf volumes obtained in Experiment 1, expressed as standard deviations, are shown in Table I and the average loaf volumes for each laboratory are given in Table II. Corresponding results from Experiment 2 are given in Tables III and IV.

TABLE I

VARIABILITY OF LOAF VOLUME, EXPERIMENT 1

	Standard deviation, *c.c.*			
	Between days (7 D.F.)		Error (98 D.F.)	
Lab.	Hand	Machine	Hand	Machine
A	5.4	8.0	16.3	13.8
B	10.6	6.0	28.2	24.4
C	13.6	9.5	23.0	22.8
D	8.5	19.3	43.0	45.2

TABLE II

MEAN LOAF VOLUME, EXPERIMENT 1

Lab.	Hand	Machine
	c.c.	*c.c.*
A	794	713
B	789	751
C	747	703
D	712	649

In Experiment 1 laboratories A and B showed significantly lower error by the machine. The differences in the other laboratories were not significant. The differences between days show no advantage for either method of manipulation. The spread between the average volumes is not reduced by use of the machine.

In Experiment 2 all the laboratories gave lower standard deviations "within days" by machine but the differences are only significant for laboratories A and B. The small number of degrees of freedom for "between days" (three) makes it impracticable to compare the standard deviations statistically but three out of the four laboratories

TABLE III

VARIABILITY OF LOAF VOLUME, EXPERIMENT 2

Lab.	Standard deviation, *c.c.*			
	Between days (3 D.F.)		Within days (56 D.F.)	
	Hand	Machine	Hand	Machine
A	6.4	3.1	17.8	10.1
B	10.0	14.8	25.0	20.4
C	18.9	6.2	26.0	22.4
D	17.2	16.1	51.7	45.2

TABLE IV

MEAN LOAF VOLUME, EXPERIMENT 2

Lab.	Hand	Machine
	c.c.	*c.c.*
A	929	791
B	936	857
C	796	757
D	761	620

gave lower results for the machine. Again, the use of the machine did not reduce the spread between laboratories.

The results show that on the whole there was slightly lower variability when the doughs were manipulated by machine, but the advantage is of no practical importance and certainly does not warrant the general adoption of the machine. This study confirms the conclusion reached in an earlier study of the Thomson moulder (Geddes, Larmour, and Malloch, 1936), that "differences in the manual manipulation of doughs during moulding are relatively unimportant in relation to the total effect of other factors causing variation between replicates."

It is interesting to note, however, that the machine showed to the best advantage in laboratory A, where the variability by both methods was strikingly lower than in the other laboratories. From this it might reasonably be concluded that when the major causes of variation have been removed, mechanical punching and moulding can be usefully employed in test baking. In laboratories where the average loaf volume is low (under 700 c.c.) it might be useful even now.

Acknowledgments

I am greatly indebted to Dr. W. F. Geddes, Dominion Grain Research Laboratory, Winnipeg; Dr. R. K. Larmour, University of Saskatchewan, Saskatoon; Dr. A. G. McCalla, University of Alberta,

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A CRITICAL STUDY OF A "PUP" SPONGE BAKING METHOD [1]

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(Read at the Annual Meeting, May 1938)

One of the criticisms often expressed against the American Association of Cereal Chemists' recommended baking procedure is that it is a straight dough method. It is frequently argued that since more of the baked goods sold on the market result from the sponge-dough process than from the straight dough process, it would be advantageous, or possibly essential, to arrange the Association baking test on a sponge-dough procedure. This contention is certainly worthy of every consideration; consequently, the following investigation was undertaken in an effort to formulate a satisfactory "pup" sponge baking method and to evaluate the results obtained.

Method

The following formula and method were adopted.

Sponge		*Dough*	
Flour	70.0%	Flour	30.0%
Yeast	1.1%	Milk powder	2.8%
Shortening	1.5%	Salt	2.0%
Malt powder, 20° L.	0.6%	Sugar	2.0%
Arkady	0.3%	Water—sufficient to yield proper dough consistency.	
Water—sufficient to yield proper sponge consistency.			
Sponge time 4 hours.			

Total flour weight was 200 g. on a 15% moisture basis. Both the sponge and dough were mixed two minutes in the Swanson mixer. The dough was fermented 20 minutes, divided into two equal portions, rounded up, allowed to rest 15 minutes, and panned. The proofing time was 55 minutes.

The sponge-dough formula adopted was purposely arranged to include the ingredients used by the average baker. This procedure is

[1] Sub-committee report, 1937–38 Committee on Standardization of Laboratory Baking.

in contrast to the A. A. C. C. baking method, the basic procedure of which utilizes a lean formula with a minimum of ingredients.

Choosing the proper sponge time is always a controversial matter; therefore, the four-hour period selected for this work was adopted, primarily because it came within the range of sponge times generally recommended, and in addition had the advantage of allowing a series of bakes to be completed within the period of a normal working day.

When undertaking to utilize the sponge-dough method to evaluate a series of flours which vary in character over the complete range encountered in the bake shop, it obviously is not a fundamentally sound procedure to attempt the use of the same flour for both sponge and dough purposes. The only justification for such a procedure must be based on the idea that the final baking results possibly would be indicative of the flour's inherent ability to produce bread under the conditions imposed. Embracing this concept invalidates the basic cause for seeking a "pup" sponge-baking method, because the A. A. C. C. procedure, admittedly, even by its most severe critics, does definitely differentiate between flours. Therefore, if a more essential question than this is not sought no useful purpose is served by deviating from the present official straight dough method. In other words the concept is that there already existed a tried and tested baking procedure; consequently, if there is any justification for a supplementary "pup" sponge method of flour testing, then the procedure should parallel closely that encountered in the baking industry. Most assuredly it would not be a sound practice to test all flours by utilizing them for both the sponge and dough. Consequently, to avoid this situation a standard topping flour of known suitability for this particular purpose was used throughout the investigation. Conversely it would be possible by this baking procedure to test the relative suitability of a flour for doughing purposes by using a standard flour for the sponge.

"Pup" Sponge-Baking Results

Figure 1 indicates the general characteristics of "pup" sponge bread baked by the method previously outlined. Bread made from three very different flour types is represented in the picture. From left to right the flour types represented are: a southwestern baker's standard patent, a spring wheat short patent, and a very strong spring wheat straight grade flour. It is evident from Figure 1 that bread of satisfactory internal and external appearance is produced.

Comparison of Sponge and Straight Dough Bread

In order to compare the results of testing flour by both the sponge-dough and straight dough methods, 24 samples of flour of various types

were collected from commercial bake shops. These flours were baked simultaneously by both methods and therefore the physical conditions as well as the ingredients used were identical. The volume of the

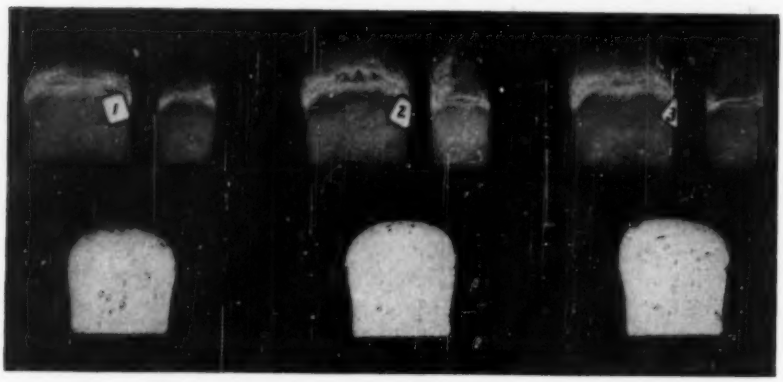

Fig. 1. Examples of "pup" sponge loaves baked from (1) southwestern baker's standard patent, (2) spring short patent, and (3) strong spring straight grade.

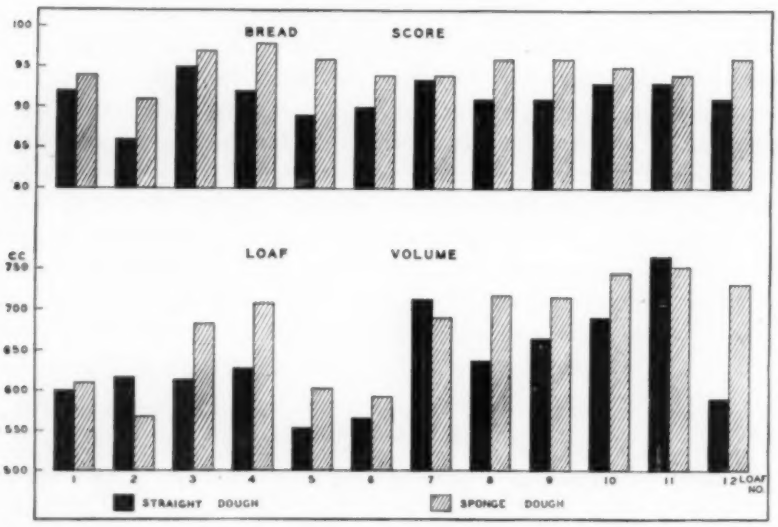

Fig. 2. Typical examples of the relationship between the bread scores and the loaf volumes of bread baked by a straight dough as compared with a sponge-dough method.

baked loaves and the bread score were used as criteria of the differences between the two methods of making bread. The loaf volumes were obtained as soon as the loaves were cool, and the bread was scored the following day. Numerical values were assigned to such internal and external characteristics as volume, symmetry, bloom, break, color, grain, and texture.

A comparison of the two baking methods, by utilizing the 24 flours previously mentioned, revealed a low but significant ($r = 0.53$) correlation between the loaf volumes and a very significant relationship between bread scores. The correlation coefficient in the latter case was $r = 0.81$. These relationships are shown graphically in Figure 2.

Discussion of Results

Loaf volume is only one of the several criteria indicating flour quality; therefore a significant relationship between the loaf volumes of bread baked from the same flour, by both the sponge and straight dough methods, is of limited importance. However, the high correlation between the bread scores when bread is made by both methods is vitally important. Obviously this indicates that both methods are applicable for the evaluation of flour quality. The question then becomes one of determining which one of the two methods, as thus conducted, yields the most valuable results.

The sponge-dough method, in the authors' opinion, is definitely a less sensitive procedure than the straight dough method. Many of the evident differences between loaves which are extremely helpful in evaluating flour baked by the straight dough process are so modified by the sponge-dough method that the tendency is toward a uniform pattern. Thus, from a testing point of view, the sponge dough method is less useful than the straight dough process, and accordingly does not warrant consideration as a substitute for the present "official" baking test.

Summary and Conclusions

A "pup" sponge baking procedure has been outlined.

Baking comparisons between "pup" sponge and "pup" straight dough methods reveal that there is a significant correlation between the loaf volumes and bread scores obtained.

The sponge-dough method, under the conditions adopted in this investigation, was a less sensitive test of flour quality than the straight dough method.

THE QUESTION OF SUGAR LEVELS IN LABORATORY BAKING [1]

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(Read at the Annual Meeting, May 1938)

Admittedly an essential feature of any *standard* experimental baking test is the establishment of rational conditions whereby all flours may be tested on an equal basis. Flours vary widely in their gas-producing potentialities, but since the measurement of this factor—which is easily controlled in industrial practice—is not the primary objective in test baking, it should be removed from the list of variables, as has been suggested by numerous workers. As recently pointed out in a detailed discussion by Landis and Frey (1936), the strict elimination of gas production as a variable factor demands a special sugar-level adjustment for each individual flour, if other factors are to be kept constant.

That a properly conducted gassing-power test, involving actual yeast fermentation, provides a far more reliable basis for the measurement of sugar requirements than does the autolytic maltose test is quite generally recognized by cereal technologists. However, recent studies by Blish and Sandstedt (1937) and by Sandstedt and Blish (1938) indicate that the control of sugar levels in doughs does not necessarily insure a corresponding control of uniformity in *rate* of gas production. These studies show that flours contain, in varying degrees, an unidentified biocatalytic "activator" (Factor M), which stimulates and influences rate of maltose fermentation by baker's yeast. This variation in quantity of Factor M seemingly accounts for the observed fact that flours having the same *maltose-producing* potentialities sometimes show significant variations in gas-production rate, and these variations frequently register conspicuously during the dough proofing period.

Since suitable means for controlling the quantity and activity of Factor M are lacking, it follows that rate of gas production is difficult, if not impossible, to control with any high degree of precision. This situation has led Sandstedt and Blish (1938) to the belief that for practical purposes the factor of variable activator content can best be handled

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by insuring an adequate sugar *supply* and then by proofing doughs to constant *height* rather than to constant *time*.

Under certain practical conditions of routine laboratory baking it is inconvenient and time-consuming to undertake to adjust all flours to precisely the same gas-production level by means of careful and accurate gassing-power tests. Indeed there are doubtless many purposes and occasions for which such precise control of conditions is quite unnecessary. The first consideration, of course, is to insure against any serious deficiency in gas production by the use of sufficient sugar in the formula. This brings up a question as to what constitutes a serious sugar deficiency, and what quantity of sugar may be regarded as excessive. Within what range can the quantity of sugar be varied without seriously complicating either baking behavior or interpretation of results? Is this range substantially the same for all flours, or does it vary significantly with different flours? In an attempt to secure practical information relative to these and associated matters some experiments were undertaken in which several commerical flours of various type and origin were experimentally baked under conditions involving different sugar levels.

Experimental

The main objective in the experiments here reported was to test the idea that, when there is no pronounced *deficiency* of sugar supply in the dough, it may be possible to vary the sugar level over a considerable range without seriously affecting loaf characteristics and interpretation of results, provided doughs are proofed to constant *height* instead of to a standard time. The experiments involved six commercial baker's flours whose origins and properties, respectively, are shown in Table I.

TABLE I

Sources and Properties of Flours

Flour origin	Protein	Maltose value	Gassing power [1]
	%	*mg.*	*mm. of mercury*
1 Pacific Coast	11.2	195	388
2 Montana	14.2	250	412
3 Kansas	12.9	335	416
4 Minnesota	12.9	287	413
5 Kansas	12.5	354	445
6 Canada	13.2	378	545

[1] Total pressure at 4 hours.

Each of the flours shown in Table I was baked, following the A.A.C.C. method, with four different amounts of added sucrose, namely, 2.5 g., 3.5 g., 4.5 g., and 5.5 g., respectively, and all doughs

were proofed to the standard time of 55 minutes. The entire program was then repeated, but all doughs were proofed to standard height instead of standard time. On the basis of preliminary tests, 9.5 cm. was selected as a suitable height. Loaf volumes for the two series of tests are shown in Tables II and III, respectively. The detailed presentation of additional baking data is omitted because such additional data would contribute nothing of importance to a rational interpretation of results.

TABLE II

LOAF VOLUMES FOR VARIOUS SUGAR LEVELS WHEN PROOFING TO STANDARD TIME (55 MINUTES)

	2.5 g. sucrose		3.5 g. sucrose		4.5 g. sucrose		5.5 g. sucrose	
Flour	Proof ht.	Vol.	Proof ht.	Vol.	Proof ht.	Vol.	Proof ht.	Vol.
	cm.	*c.c.*	*cm.*	*c.c.*	*cm.*	*c.c.*	*cm.*	*c.c.*
1	8.8	535	9.0	545	9.3	595	9.5	545
2	9.8	630	10.1	625	10.0	625	10.1	670
3	8.8	525	9.0	535	9.2	345	9.3	545
4	8.9	570	8.9	560	9.2	570	9.3	615
5	9.0	560	9.0	565	9.1	570	9.1	580
6	10.5	605	10.2	570	10.0	570	10.0	575

TABLE III

LOAF VOLUMES FOR VARIOUS SUGAR LEVELS WHEN PROOFING TO STANDARD HEIGHT (9.5 CM.)

	2.5 g. sucrose		3.5 g. sucrose		4.5 g. sucrose		5.5 g. sucrose	
Flour	Proof time	Vol.	Proof time	Vol.	Proof time	Vol.	Proof time	Vol.
	min.	*c.c.*	*min.*	*c.c.*	*min.*	*c.c.*	*min.*	*c.c.*
1	69	575	62	580	58	575	59	580
2	51	590	51	600	48	570	45	590
3	67	560	64	555	59	570	57	580
4	62	590	65	570	60	590	60	605
5	62	590	62	585	60	580	54	580
6	43	560	49	550	50	560	50	550

The data in Tables II and III do not suggest the necessity for a high degree of precision in the adjustment of sugar levels in experimental baking. Varying the quantity of added sugar over a range of from 2.5 to 5.5 g. for the most part produced no significant variations in loaf properties other than crust color. Variations in responses to different sugar levels were surprisingly small whether proofed to constant height or to constant time, with perhaps slightly less variation when proofed to height.

The nature of the volume response to the two methods of proofing was not the same for all flours. Thus flour No. 1 gave significantly

larger volumes when proofed to height than when proofed to time, as did flour No. 3. With flour No. 2, however, the effect was distinctly the opposite. The range of variation from one flour to another was less when proofed to height than when proofed to time. The flours show a considerable range of variation as to time required to proof to standard height, regardless of sugar level. This probably was due at least in part to variations in Factor M.

Varying Sugar Levels with Shortening

The experiments represented by the data in Tables II and III were repeated, using in all bakes 2% of shortening (Crisco) in the dough formula. This study was deemed advisable in view of the fact that shortening is frequently observed to be an important factor influencing flour responses and flour behavior. The influence of shortening on the loaf-volume responses to additions of varying quantities of sucrose is shown in Tables IV and V.

TABLE IV

LOAF VOLUMES FOR VARIOUS SUGAR LEVELS WHEN PROOFING TO STANDARD TIME (55 MINUTES) WITH 2% OF SHORTENING

Flour	2.5 g. sucrose Loaf vol.	3.5 g. sucrose Loaf vol.	4.5 g. sucrose Loaf vol.	5.5 g. sucrose Loaf vol.
	c.c.	*c.c.*	*c.c.*	*c.c.*
1	575	565	630	610
2	645	650	650	710
3	560	570	605	605
4	600	605	660	670
5	595	605	630	635
6	670	670	640	640

TABLE V

LOAF VOLUME FOR VARIOUS SUGAR LEVELS WHEN PROOFING TO STANDARD HEIGHT (9.5 CM.) WITH 2% OF SHORTENING

Flour	2.5 g. sucrose Loaf vol.	3.5 g. sucrose Loaf vol.	4.5 g. sucrose Loaf vol.	5.5 g. sucrose Loaf vol.
	c.c.	*c.c.*	*c.c.*	*c.c.*
1	595	615	635	635
2	655	635	655	670
3	580	605	580	620
4	660	670	685	695
5	640	630	645	660
6	630	630	650	660

The data in Tables IV and V indicate that the use of shortening tended to increase loaf volumes at all sugar levels. It is also apparent that the use of shortening favored a marked tendency toward positive volume response to *higher sugar levels* when the doughs were proofed

to standard time. This was not evident for Nos. 2 and 6, but for 1, 3, 4, and 5, the volumes were significantly higher at the two higher sugar levels than at the two lower ones.

Proofing to standard *height*, however, tended to equalize loaf volumes over the entire range of sugar levels.

Shortening is used in commercial practice. Since it is a factor influencing flour responses, it should perhaps be used in experimental baking.

The above experiments support the contention of Sandstedt and Blish (1938) that the safest procedure is to use a liberal quantity of sugar and proof to standard *height*. This should reduce the chances of unjust discrimination against flours that tend to proof slowly under conditions of the straight dough method, but which would not be of any serious disadvantage in commercial baking where the sponge method predominates. The possibilities in this connection are photographically illustrated in Figure 1.

Fig. 1. Overcoming the effect of different sugar levels by proofing to height instead of to time.

The loaves in Figure 1 were all baked from the same flour. Loaves O and P were baked with 2½ and 5 g. of sugar, respectively, and proofed to the same height. Loaves Q and R were also baked with 2½ and 5 g. of sugar, respectively, but proofed to the same *time*. This method of proofing unjustly penalized Q both in volume and in oven-spring, as compared with O, which was proofed to *height*. The higher sugar level in R practically offsets the slowness of proof. When proofed to height, however, there is no particular advantage in favor of the higher sugar level.

These considerations are further illustrated photographically in Figure 2. Loaves F, G, and H were baked from three different flours with 2½ g. of sugar, and proofed for 55 minutes. F is a slow proofing flour (proofs to 9.0 cm. in 55 minutes), while G and H are rapid proofers (proof to 11.5 cm. in 55 minutes). I, J, and K are the same flours, baked as before but proofed to the same height (9.5 cm.).

Flour F, a slow proofer, gave a much better oven-spring when proofed to height (see I) than when proofed to the standard time. As proofed to time (F) it might be considered as "underproofed." Flour H, a rapid proofer, when proofed to time, showed little oven-spring and was obviously "overproofed." This, also, was corrected by proofing to height (9.5 cm.) as shown in loaf K. A similar condition exists in the case of the flour represented by loaves G and J.

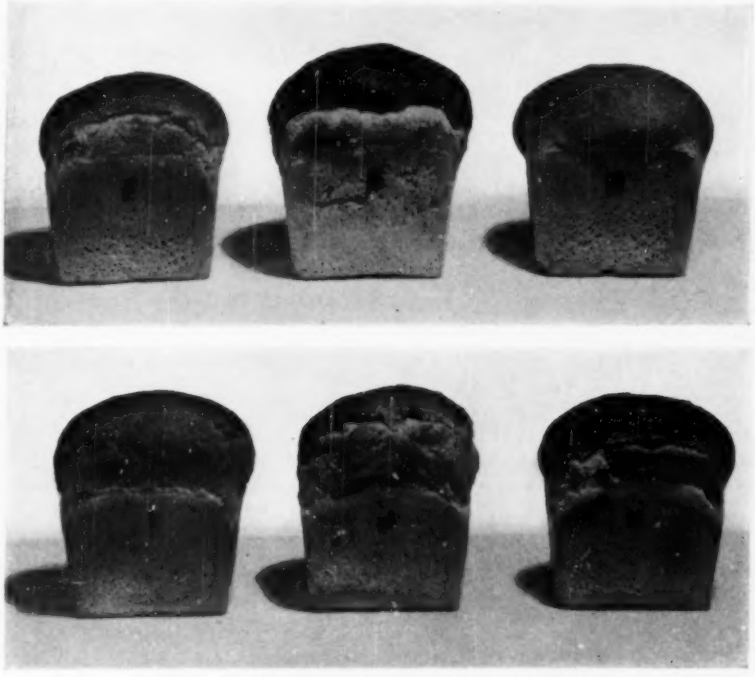

Fig. 2. Proofing to standard time vs. standard height.

From the experiments herein reported it is possible to conclude that in most experimental baking the safest all-round procedure is to use shortening, to adopt a sugar level somewhere in the range afforded by the addition of 4.5 to 5 g. of sugar for commercial flours of average gassing power, and to proof to constant height rather than to constant time. A height of 9.5 cm. has been used preferentially in these studies, but some other height might, as a result of additional study, be established as more suitable. This will perhaps depend upon the type of pan used or eventually adopted.

Summary and Conclusions

Six commercial bakers' flours of varying types, but all having at least *average* gassing powers, were experimentally baked at different

sugar levels. The effect of varying sugar levels was observed with reference to proofing to standard time as against proofing to constant height. In these connections the influence of shortening was also noted.

Aside from crust color, the effects on loaf properties produced by variations in added sucrose over a range of 2.5 to 5.5 g. were surprisingly unimportant, when shortening was omitted from the dough formula. With shortening, however, a positive volume response was effected in the higher sugar levels when doughs were proofed to standard time, but this effect was minimized when the proofing was to constant height.

In establishing a rational basis for interpretation of experimental baking tests, the use of shortening, of relatively high sugar levels, and proofing of the doughs to constant height are features which appear to offer the safest all-round procedure.

A standard or constant sugar level does not insure a constant rate of gas production.

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EFFECT OF ADDING ALPHA- AND BETA-AMYLASES TO DOUGHS[1]

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(Received for publication June 22, 1938)

Numerous investigations of the importance of the diastatic enzymes to fermentation and gas production in doughs have been conducted, and Landis and Frey (1936) have discussed these thoroughly in an excellent historical review. The majority of the studies have involved malt extracts, malted wheat flours, and commercial malt diastase preparations. Such preparations contain both alpha- and beta-amylases in various proportions. Alpha-amylase, the dextrinizing enzyme, is generally considered most important in relation to diastatic activity. Recently the work of Blish, Sandstedt, and Mecham (1937) indicates that a "raw starch factor" is always found

[1] Paper No. 1625, Journal Series, Minnesota Agricultural Experiment Station.

present in alpha-amylase preparations and is of importance in raw-starch hydrolysis. Beta-amylase, the saccharifying enzyme, is usually found in abundance in wheat flours. This enzyme attacks the labile

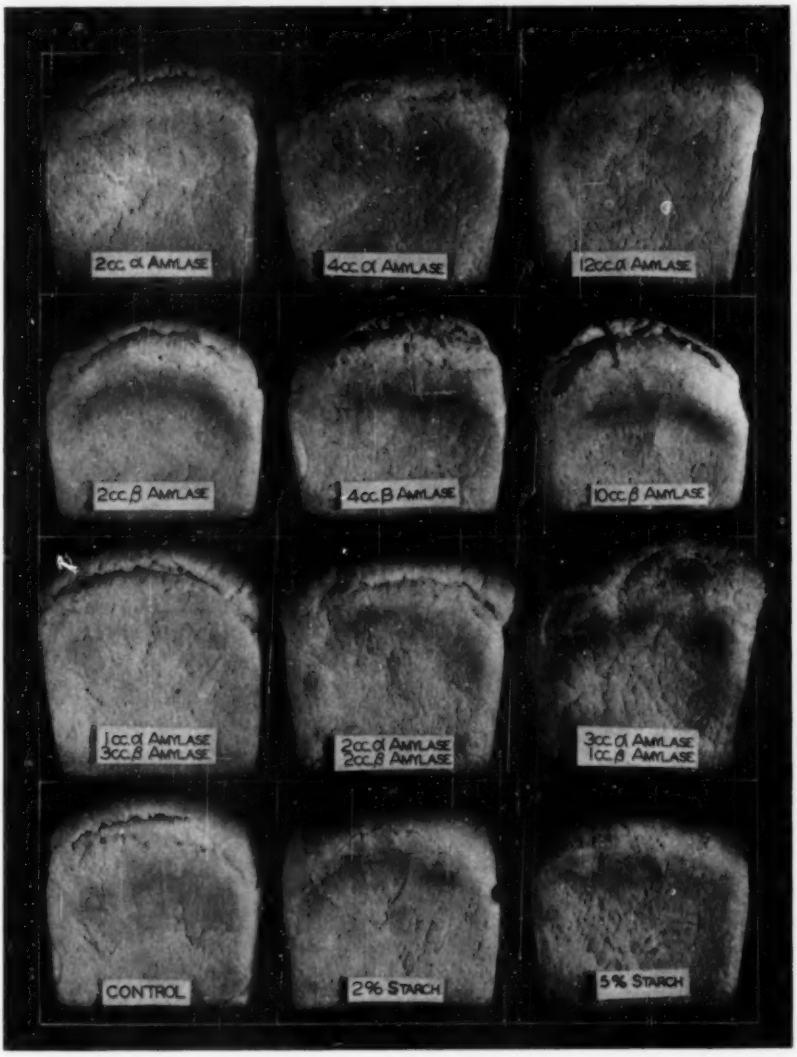

Fig. 1. Exterior of loaves made with various amounts and combinations of amylases and finely ground wheat starch. Fermentation time was three hours.

portions of the starch, and also the dextrins produced by alpha-amylase preparations. Thus the increase in diastatic activity upon overgrinding flours is mainly due to the action of beta-amylase on the fine fragments of broken-down starch granules.

The purpose of this work was to study the effect of alpha- and beta-amylases added to doughs, instead of diastase preparations with

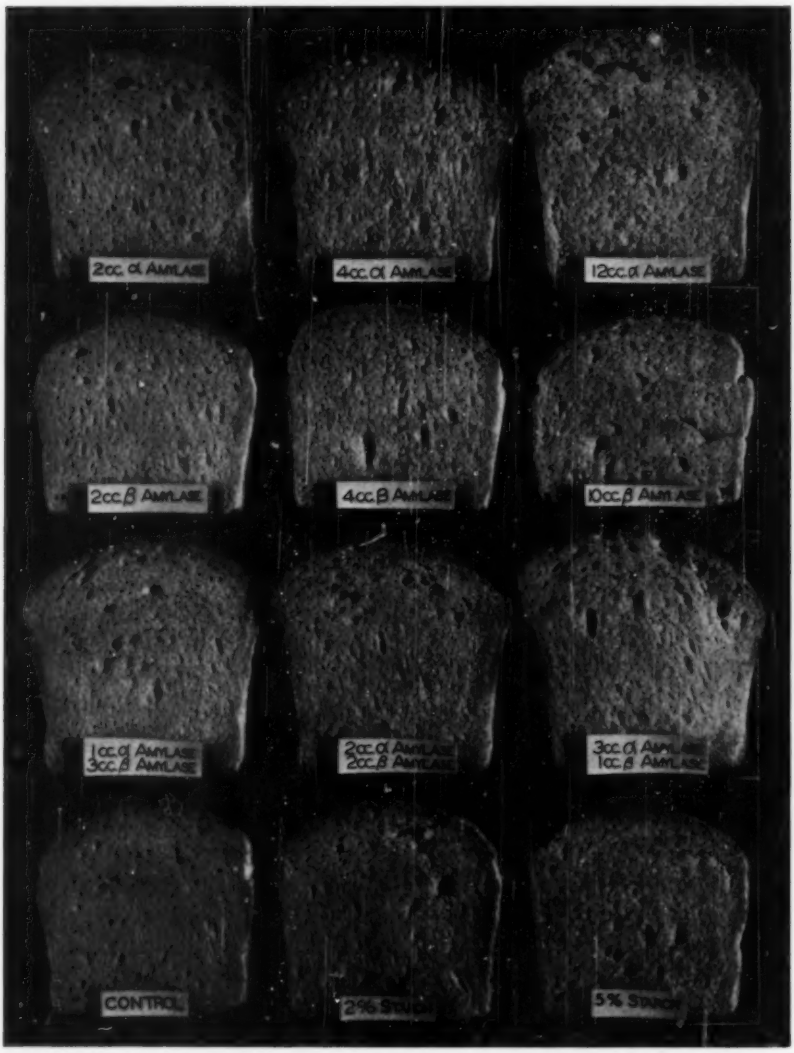

Fig. 2. Interior of loaves made with various amounts and combinations of amylases and finely ground wheat starch. Fermentation time was three hours.

appreciable amounts of both enzymes, and to obtain a pictorial record of the effect of these amylases on the bread when used in various amounts.

Experimental

The alpha-amylase preparation was made by Ohlsson's (1926) technique. An extract from germinated wheat was heated to 70° for 15 minutes to inactivate most of the beta-amylase present.

The beta-amylase preparation was an extract from normal wheat which was acidified by HCl to pH 3.3 for 15 minutes at 0° (Ohlsson, 1926) and then brought to pH 6.0 by secondary sodium phosphate.

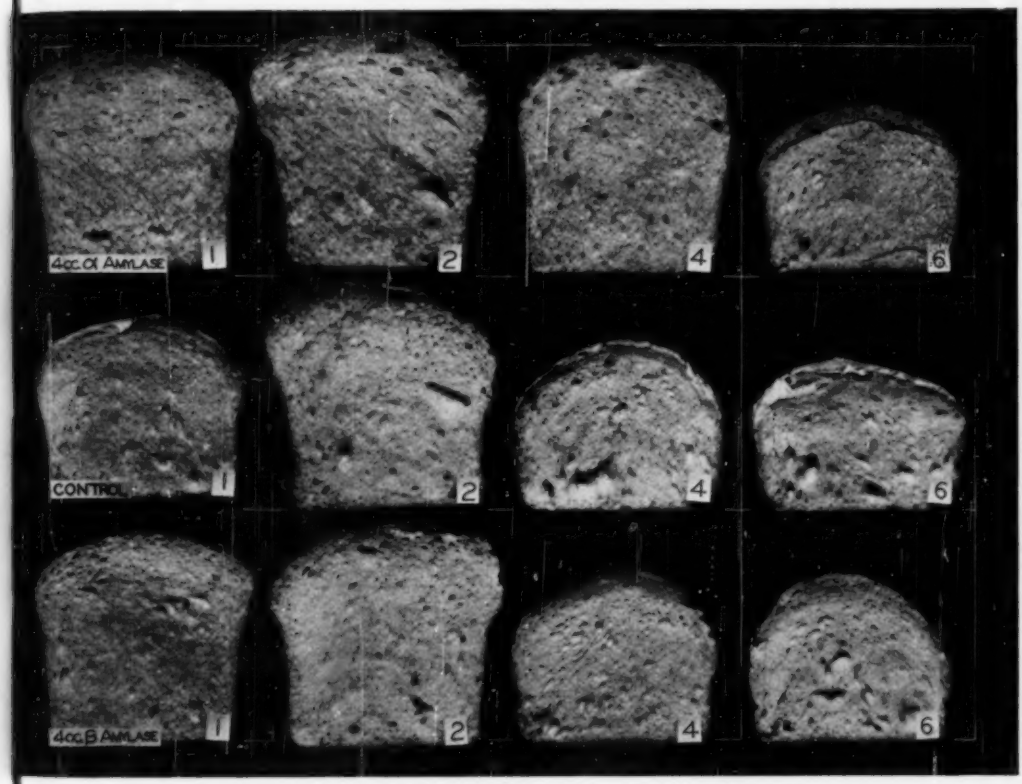

Fig. 3. Interior of bread made with 4 cc. of alpha- and beta-amylase in doughs with 1, 2, 4, and 6 hours of fermentation.

The extracts were kept at 0° until used. A small portion of the germinated wheat extract was used in precipitating alpha-amylase by adding alcohol to 60% concentration. Some of the normal-wheat extract was used also to precipitate beta-amylase at 80% concentration of alcohol. The precipitates were washed with absolute alcohol and dried under vacuum.

The alpha-amylase preparations exhibited high dextrinizing activity

on soluble starch, while the beta-amylase preparations showed no dextrinizing action on soluble starch but produced rapid saccharification.

The flour used for all baking tests was of medium strength, containing about 11% crude protein, and having a diastatic activity of 220 Rumsey units. The straight-dough procedure was used, and A. A. C. C. 100-gram loaves were baked. Each loaf contained the following ingredients:

100 g. flour	62% adsorption (includes enzyme solutions)
1% salt	no sugar
3% yeast	no shortening

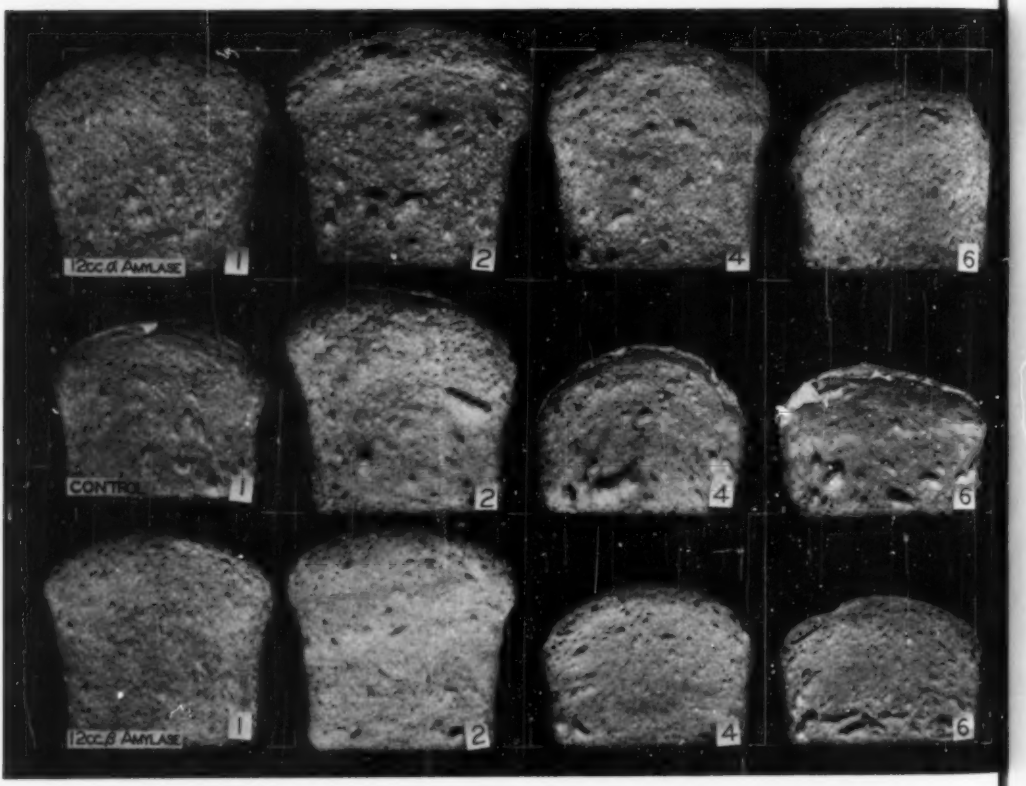

Fig. 4. Interior of bread made with 12 cc. alpha- and beta-amylase in doughs with 1, 2, 4, and 6 hours of fermentation.

The doughs were mixed for two minutes in the Hobart-Swanson mixer. The fermentation temperature was 30°. In one series of doughs the fermentation time was 3 hours, and in another series 1, 2, 4, 6, and 8 hours respectively. The pan proof was 55 minutes at 30°, and the loaves were baked for 25 minutes at 230°.

Finely pulverized wheat starch was added at 2% or 5% levels to certain doughs, replacing a like amount of flour. Thus the amount of substrate readily available for beta-amylase activity was increased in these instances. Sugar-free [2] doughs were used, since the presence of added sugar might obscure the effect of the amylases.

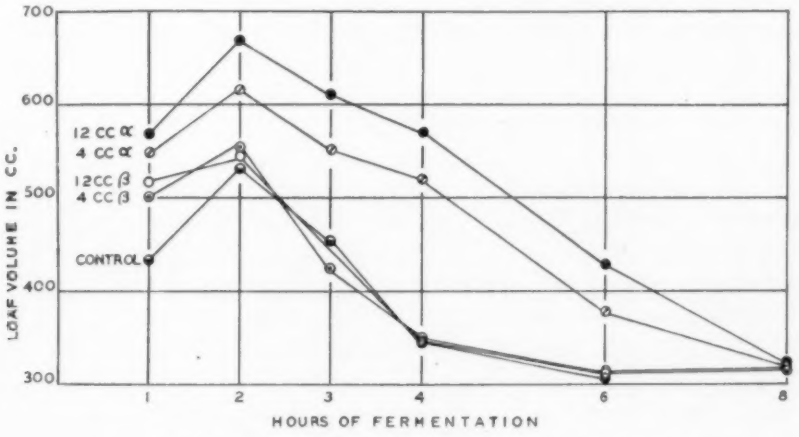

Fig. 5. The effect on loaf volume of the addition of alpha- and beta-amylase extracts to sugar-free doughs.

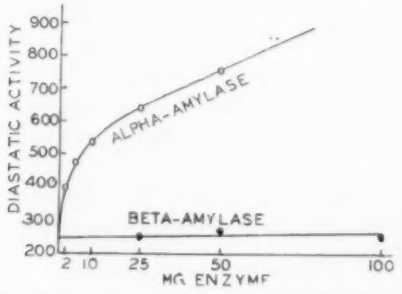

Fig. 6. Effect of the addition of alpha- and beta-amylase preparations upon the diastatic activity of flour, recorded in Rumsey units. The diastatic activity of the original flour was 245 R. units. Additions of enzymes are in terms of milligrams of the dry preparation per 5 g. flour.

Table I shows the effect on loaf volume and crust color of bread made with various additions, alone and in combination, of the enzyme extracts and also with the ground wheat starch added. Figures 1 and 2 show the exteriors and interiors, respectively, of the loaves produced.

Another series of doughs involved variations in fermentation time. The doughs accorded the longer fermentation of 2 to 8 hours were punched at every hour interval after mixing. Two dosages of enzymes were added, namely 4 c.c. and 12 c.c. of each enzyme extract.

[2] Meaning that no sugar was used in the formula.

TABLE I

BAKING TESTS WITH VARYING AMOUNTS AND COMBINATIONS OF AMYLASES AND GROUND STARCH

Three hours' fermentation time before panning—Figures 1 and 2 show the appearance of the loaves

Amount and kind of amylase solution or ground starch added			Loaf volume	Color of crust
Alpha-amylase		Beta-amylase		
c.c.		*c.c.*	*c.c.*	
0	(control)	0	454	++
2		0	543	+++
4		0	553	++++
12		0	610	++++
0		2	418	++
0		4	423	++
0		10	395	++
1		3	549	+++
2		2	550	+++
3		1	608	++++
2% ground wheat starch			445	+++
5% ground wheat srarch			450	++++

++ = yellow.
+++ = light-brown.
++++ = brown.

The results are recorded in Table II, in terms of loaf volume and crust color. Interiors of the corresponding loaves are depicted in Figures 3 and 4. Figure 5 records volume in c.c. of loaves baked from control doughs and from doughs that were fermented for varying intervals, and prepared with two proportions of alpha- and beta-amylase.

Amylases precipitated by alcohol in the manner already described were added in varying quantities to a flour similar to that used in these

TABLE II

THE EFFECTS OF DIFFERENT AMOUNTS OF ALPHA- AND BETA-AMYLASE WITH VARIOUS FERMENTATION TIMES UPON LOAF VOLUME AND CRUST COLOR

(See Figures 3 and 4.)

Fermentation time	Alpha-amylase				Beta-amylase				Control	
	4 c.c.		12 c.c.		4 c.c.		12 c.c.			
	Loaf volume	Crust color	Loaf volume	Crust color	Loaf volume	Crust color	Loaf volume	Crust color	Loaf volume	Crust color
Hrs.	*c.c.*		*c.c.*		*c.c.*		*c.c.*		*c.c.*	
1	548	+++++	567	+++++	503	+++++	518	+++++	484	+++
2	615	++++	608	+++++	553	+++	545	+++	532	+++
3	553	++++	610	++++	423	++	—	—	454	++
4	520	++++	572	++++	350	++	345	++	344	+
6	381	+++	429	+++	315	++	314	++	306	+
8	319	+	324	+	315	+	316	+	—	—

\+ = pale-yellow.
++ = yellow.
+++ = light-brown.
++++ = brown.
+++++ = dark-brown.

baking experiments. This flour had an original diastatic activity of 245 Rumsey units. The effects of these additions, here recorded in terms of milligrams of the dry enzyme preparation per 5 grams of flour, are indicated graphically in Figure 6, again in Rumsey units.

Discussion

The data recorded in Table I and Figures 1 and 2 indicate that the addition of a small amount (4 c.c.) of alpha-amylase preparation to a normal, medium-strength flour dough improved the crust color, grain, and volume of the resulting loaves. The addition of a larger quantity of alpha-amylase (12 c.c.) produced a larger loaf but at the expense of grain and texture, which were inferior. The addition of beta-amylase in various amounts produced in all instances a poorer loaf of bread than the control. With increase in beta-amylase there was a decrease in loaf volume, paler crust color, poorer interior, and a greater tendency to give "shell top" loaves. In the various enzyme combinations used there was an improvement of the bread as alpha-amylase was increased up to 4 c.c. Beta-amylase appeared to be sufficient in the normal flour and the addition of beta-amylase had no improving effect. This is in accord with Andrews and Bailey (1934), who found that normal flours contain sufficient amounts of beta-amylase. The addition of finely pulverized wheat starch increased the diastatic activity of the dough as shown by the deeper crust colors, but the grain and texture were impaired, and there was no increase in loaf volume. The "break" was improved slightly. Alsberg and Griffing (1925) found that fine grinding of flours increases the diastatic activity but injures the baking quality.

Figures 3 and 4 show that the addition of small amounts of alpha-amylase (4 c.c.) improved the loaf volume considerably at the various fermentation times. Three times as much alpha-amylase (12 c.c.) increased the loaf volume slightly more, but inferior grain and texture resulted. Observations made are in agreement with those of Kozmin (1933), that flours made with the addition of too much sprouted wheat produce bread with defective qualities. The crumb loses its elasticity and dryness and appears damp and poorly baked. This is probably due to the dextrination of the starch by the enzymes beyond desirable limits, leaving insufficient unattacked starch for binding of the water in the dough during the baking process.

The addition of small or large amounts of beta-amylase did not improve the doughs, as shown in Figure 5, but an increased tendency to form "shell top" loaves was noticed after two hours' fermentation of the dough. When pulverized wheat starch was added the loaves

did not develop a shell top, although of about the same volume as loaves made with beta-amylase.

Figure 6 indicates that normal flours contain sufficient quantities of beta-amylase and that the addition of more beta-amylase does not result in a useful increase in the diastatic activity of the flour. Only small quantities of alpha-amylase or sprouted-wheat enzymes are necessary to produce a considerable increase in diastatic values of flours, since alpha-amylase, and its accompanying raw starch factor as explained by Blish and coworkers, readily attacks the starch in the flour to produce dextrin materials which are then saccharified by beta-amylase.

Care must be exercised in diastating doughs, and although large loaves are produced by increased diastatic activity, there is danger in overdiastating doughs with resulting inferior crumb, grain, and texture. This might be one reason for the sogginess of the crumb of some commercial bread found in certain markets.

Summary

Alpha-amylase in small amounts added to sugar-free doughs improved the bread considerably, but larger amounts of alpha-amylase resulted in bread having inferior crumb, grain, and texture. Beta-amylase in small or large amounts did not improve the bread, and this enzyme appeared to be present in the flour in sufficient quantities.

Addition of finely pulverized wheat starch to the flour improved the crust color and "break" of the bread but had a detrimental effect on the grain and texture and produced no increase in volume.

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FURTHER INVESTIGATIONS INTO THE NATURE OF THE ACTION OF BROMATES AND ASCORBIC ACID ON THE BAKING STRENGTH OF WHEAT FLOUR

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(Read at the Annual Meeting, May, 1938)

Considerable interest and discussion have been evoked by the author's papers (1935, 1935a) in which it was shown that oxidizing agents (especially potassium bromate), which act as flour improvers, inhibit the action of certain proteolytic enzymes; a theory was proposed that the pronounced improving effect of these agents on baking characteristics is to be ascribed to this inhibitory effect. Subsequently, several authors have published on this question and while several agree with the writer's theory, some others are not in accord, considering the author's interpretation of his experimental material (the correctness of which has not been contested) to be wrong. It is the purpose of the present paper to summarize the theory and its experimental basis, to examine critically a paper published by other investigators who interpret their experimental data as opposed to the writer's views, and to present additional supporting experimental evidence.

The Theory and its Experimental Basis

The experiments published in the author's paper (1935) comprised the autolysis of flour suspensions containing small quantities of the various salts under study under fixed conditions and determining the quantity of nitrogen present in the aqueous extract. The presence of flour improvers such as potassium bromate and iodate during the autolysis of the flour-water suspensions depressed the nitrogen content of the extract, whereas potassium chlorate, which is not a flour improver, was without influence; moreover these depressions were greatly accentuated when the proteolytic activity of the flour being extracted was artificially increased by the addition of papain. Similarly, potassium bromate and iodate, but not potassium chlorate, inhibited the proteolytic activity of papain and the proteases of wheat flour, wheat germ, pineapple, and liver when acting on gelatin. Ascorbic acid, which is known to reduce the activity of plant proteinases of the papain group, was shown to be a good flour improver.

Another series of experiments published by Jørgensen (1935a) showed that the "bromate-depression," *i.e.*, the reduction in nitrogen solubility as a result of the presence of potassium bromate, is greater when the flour extractions are conducted in the presence of either compressed or dried baker's yeast; however, yeast was without influence on the bromate depression if the flour-proteinases were first destroyed by heat treatment of the flour. Nevertheless, the proteins of such heat-treated flour were not changed in such a way by heating that they could not readily be rendered soluble by papain and pineapple proteinase. Glutathione was found to exert an effect similar to that of yeast on the bromate depression; an aqueous extract of dried baker's yeast, free from yeast cells, gave a very strong nitro-prusside reaction showing that glutathione or similar compounds are given off from the dried yeast to the dough in bread-making.

On the basis of the experiments published in the first-mentioned paper the author (1935) concluded that oxidizing agents such as potassium bromate, which exert an improving effect on baking properties, owe their action to their inhibitory effect on the wheat-flour proteinases which, if allowed to act on the gluten at their normal rate, may diminish baking strength. While excessive proteolytic activity is detrimental, on the other hand, if it is too low, the gluten is "unripe." There is thus a certain optimum proteolytic activity which explains the fact that baking strength may be destroyed by an excessive dosage of oxidizing agents which in smaller amounts act as improvers. It was, however, stated to be an open question whether the very strong over-treatment which for example iodates may produce, is due exclusively to excessive inhibition of the flour proteinases.

On the basis of the experiments published in the last-mentioned paper the author (1935a) concluded that the proteolytic activity in doughs containing yeast is due to the *activation* of the flour proteinases by yeast and is *not* a result of the yeast itself secreting proteinases into the dough. In the instance of compressed yeast the mechanism of this activation is an open question, but with dried baker's yeast the activation is, in part at least, due to the yeast's giving off a proteinase activator, such as glutathione, to the dough. This stimulating effect of the yeast on the flour proteinases explains the well-known fact that potassium bromate has a greater effect on doughs to which yeast has been added.

Corroboration of the Theory

Balls and Hale (1936) have shown that chlorine reduces flour proteolytic activity and, quite independently of the author, conclude that there is an optimum proteolytic activity for satisfactory ripening

of the gluten. Usually there is an excess and the beneficial effects of bleaching or natural aging are due to a diminution of proteolytic activity brought about by oxidation of the activator of the flour proteinase. They also conclude that similar effects are produced when the oxidant is added to the dough as a bread improver. The author's theory is also strongly supported by the experiments of Flohil (1936) and the investigations of Elion (1937) and Melville and Shattock (1938).

The Work of Read and Haas

On the other hand, Read and Haas (1937), who repeated certain of the extraction studies of the author, secured results which they interpret as opposed to this theory and it is necessary critically to examine their experimental data. While these workers used a somewhat different technique, the flour-water ratio, namely 25 g. flour and 100 c.c. liquid, was the same as employed by the present author Their extraction results for different quantities of potassium bromate, with normal wheat flour and also flour in which the proteolytic activity had been artificially increased by papain or pineapple juice, are presented in Table I.

TABLE I

EXTRACTION TESTS WITH POTASSIUM BROMATE

(From Read and Haas, Cereal Chem. **14**: 753)

Extracted material	Mg. $KBrO_3$ added to extraction mixtures					
	0	0.25	0.5	1	50	100
	Mg. N in 20 c.c. of extract					
25 g. flour	14.3	14.3	14.3	14.3	13.6	13.5
25 g. flour + 50 mg. papain	86.5	86.2	85.1	81.0	29.1	23.4
25 g. flour + 10 mg. papain	43.0	43.0	42.1	—	16.7	15.3
25 g. flour + 5 drops of raw pineapple juice	46.3	46.2	43.6	43.2	16.9	—

On the basis of these results, Read and Haas (1937) conclude that the inhibitory effect of potassium bromate is evidenced only when it is added in amounts greatly in excess of those used in actual baking. The author, however, is of the opinion that their results actually confirm his theory. Certainly, in the instance of the tests where 50 mg. papain and pineapple juice were employed, 0.5 mg. potassium bromate (*i.e.*, 2 g. per 100 kg.) effected an appreciable reduction in nitrogen solubility and this quantity cannot be considered excessive. With wheat flour alone, 50 mg. of bromate definitely reduced the quantity of nitrogen dissolved, and while no effect is shown by the lesser quantities employed, the author believes that these smaller

amounts also produce a reduction but it is so slight as to escape detection under the conditions of such tests. It is believed that each increment of potassium bromate paralyzes a definite percentage of the total proteolytic activity and as this total is very small under the testing conditions employed, the relatively slight reduction brought about by the addition of small amounts of potassium bromate corresponds to an inappreciable decrease in nitrogen solubility. The correctness of this explanaton is supported by the results of experiments presented later in this paper.

Read and Haas (1937) consider that reduction in the amount of dissolved nitrogen in the instance of normal flour cannot be ascribed to proteinase inhibition but must be explained otherwise, such as an electrolytic effect on the flour colloids. Moreover, they point out that this reduction is not evidenced until a 50-mg. dosage is used, an amount which is two hundred times greater than employed in bakery practice and they accordingly believe there is no connection between the reduction in dissolved nitrogen and the action of bromate on baking properties.

In reaching this conclusion, Read and Haas have failed to take two important points into consideration. First, the flour-water ratio in these extraction tests is 1 : 4, whereas in a fermenting dough it is approximately 1 : 0.6; consequently, in such tests the bromate is acting in a concentration only about one-seventh of that in fermenting doughs containing the same quantity of bromate. For this reason alone, larger amounts would be required to produce corresponding effects. Secondly, in a dough, *yeast* is present which activates the flour proteinases; hence the amounts of bromate necessary to show a reduction in nitrogen solubility in extraction tests without yeast cannot be directly compared with the quantities employed in bread doughs.

Extraction Tests with Potassium Bromate

That the presence of yeast greatly reduces the quantity of bromate required to reveal a reduction in nitrogen solubility is proved by the following experiments. Twenty-five grams of flour, commercially milled in Denmark from Manitoba No. 1 Northern wheat and containing 15.1% moisture, 2.45% nitrogen, and 0.58% ash, was extracted for 2 hours at 35° C. with an extraction mixture consisting of 75 c.c. water, 25 c.c. Sorensen phosphate mixture (pH 5.8), plus additions of potassium bromate. In the tests involving yeast, 0.5 g. ordinary baker's yeast (De Danske Spritfabrikker, Ltd., Copenhagen) was employed.

The extraction technique, which in such tests must be *well-defined*, was as follows: the extractions were carried out in 130 c.c. rubber-

stoppered centrifuge bottles; a large electrically-heated and regulated water thermostat was fitted with 12 holders mounted on a longitudinal axis and the extraction tests were carried out under water at a rotation speed of 25 r.p.m. In the tests involving yeast, the stoppers were tied in. After extraction, the bottles were centrifuged for 10 minutes in an Angle Separator (Stilles, Ltd., Stockholm) and the supernatant liquid filtered through S. and S. No. 589 filter paper; the extracts with yeast were filtered twice. The nitrogen in 20 c.c. of the filtrates was determined by the Kjeldahl method.

TABLE II

EXTRACTION TESTS WITH POTASSIUM BROMATE

(Author's Experiments)

Extracted material	Mg. $KBrO_3$ added to extraction mixtures					
	0	5	10	25	50	100
	Mg. N in 20 c.c. of extract					
25 g. flour	16.2	16.2	15.9	15.7	15.6	15.5
25 g. flour + 0.5 g. baker's yeast	20.1	18.9	18.6	18.0	18.1	17.3

The results, recorded in Table II, show clearly that 25 mg. potassium bromate reduces the amount of dissolved nitrogen when flour alone is extracted but when yeast is added the proteolytic strength is so much increased that a larger reduction is obtained with only 5 mg.; obviously, even smaller amounts would produce a distinct effect. When it is considered that this quantity of bromate is acting only in approximately one-seventh of the concentration which exists in actual fermenting doughs, it is clear that "bromate depression" is evidenced by quantities corresponding to those used in baking practice; in the Scandinavian countries, 1 to 5 g. bromate is employed for 100 kilos of flour.

Extraction Tests with Ascorbic Acid

According to the author's theory, ascorbic acid acts as a bread improver, because it, like potassium bromate and potassium iodate, also inhibits proteolytic activity. Read and Haas (1937) have carried out extraction tests with ascorbic acid similar to those they conducted with potassium bromate; their results, reproduced in Table III, are seemingly contrary to the writer's theory, since ascorbic acid produced a slight increase in water-soluble nitrogen.

It may easily be shown, however, that these results cannot be used as an argument against the theory. It is noteworthy that, while Read and Haas (1937) point out that 100 mg. potassium bromate is

TABLE III

EXTRACTION TESTS WITH ASCORBIC ACID

(From Read and Haas, Cereal Chem. **14**: 753)

Extracted material	Mg. N in 20 c.c. of extract
25 g. flour	14.1
25 g. flour + 100 mg. ascorbic acid	14.2

four hundred times greater than the quantity employed in baking, this is the very (and only) amount of ascorbic acid which they used in their ascorbic-acid extraction tests; yet in practice this chemical is used in similar amounts to potassium bromate, namely, 1 to 2 g. per 100 kilos of flour.

To examine further the behavior of ascorbic acid, the author carried out a series of extraction tests with increasing amounts of ascorbic acid, using the same flour and technique as described for the bromate tests. In these experiments, pH was determined and in certain of the tests sodium hydroxide was added to prevent a change in pH through the addition of ascorbic acid. The results are summarized in Table IV.

TABLE IV

EXTRACTION TESTS WITH ASCORBIC ACID

Series	Material extracted	Extract	Mg. ascorbic acid added to extraction mixtures					
			0	5	10	25	50	100
1.	Flour 25 g.	N in 20 c.c. (mg.)	16.2	15.6	15.6	15.7	16.0	17.6
		pH [1]	6.00	5.97	5.96	5.90	5.84	5.60
2.	Flour [2] 25 g.	N in 20 c.c. (mg.)	16.2	15.6	15.5	15.5	15.7	15.8
		pH [1]	6.01	6.00	6.01	6.00	6.02	6.01
3.	Flour 25 g. plus baker's yeast, 0.5 g.[2]	N in 20 c.c. (mg.)	20.1	18.4	18.6	18.4	18.7	

[1] Determined at 22° C. by the glass electrode.
[2] 0.038 c.c. N/10 NaOH was added per mg. ascorbic acid.

From the first series of experiments, it will be noted that, in agreement with Read and Haas, the addition of 100 mg. ascorbic acid increases the water-soluble nitrogen; however, the smaller amounts do not produce such an increase and, in fact, there is a distinct indication that the 5, 10, and 25 mg. additions result in a decrease. The pH values show clearly that the use of 100 mg. ascorbic acid lowers the pH of the extract from 6.00 to 5.60 and this appreciable decrease in pH

is the reason for the higher nitrogen content of this particular extract. A suitable addition of hydrochloric acid will, in itself, increase the water-soluble nitrogen and, consequently, this is a simple pH phenomenon and not a specific effect of ascorbic acid. In practice, 1 to 2 g. ascorbic acid per 100 kilos are used and these amounts do not produce a measurable decrease in pH. For example, a dough consisting of 280 g. flour, 4 g. salt, and 160 c.c. water was found by direct measurement with the glass electrode to be pH 5.95 (23° C.) and a similar dough containing 5.6 mg. ascorbic acid (equivalent to 2 g. per 100 kilos) showed precisely the same value.

In order properly to study the influence of ascorbic acid on proteinase activity by extraction tests, it is obviously necessary to eliminate the pH effect. This has been done in the second and third series of experiments recorded in Table IV, and under these conditions, it is seen that ascorbic acid reduces the water-soluble nitrogen, the reduction being much more pronounced when yeast is also present. These extraction tests thus afford excellent support for the author's theory that ascorbic acid acts as a baking-strength improver because it inhibits the flour proteinases.

The question why ascorbic acid, which is a reducing agent, produces an effect similar to that of potassium bromate, an oxidizing agent, remains to be answered. In a series of patent applications,[1] the author has advanced the view that ascorbic acid may possibly act as an oxygen carrier, absorbing oxygen from the atmosphere and the oxidized acid then imparting the oxygen to the flour, thereby inactivating the flour proteinases. This hypothesis has recently been supported experimentally by Melville and Shattock (1938).

The Nature of Wheat Flour Proteinases

As is well known (see Grassman and Schneider, 1936), proteinase enzymes may be classified into three main groups: (1) the pepsin group, optimum pH 2, approximately; (2) the papain-cathepsin group, optimum pH 4 to 7; and (3) the trypsin group, optimum pH 8, approximately.

Balls and Hale (1935, 1936) maintain that the flour proteinases active in the baking process belong to the papain group. The author's investigations (1935) show an extraordinarily close analogy between papain and the wheat proteinases; Flohil (1936) also concludes that the wheat germ proteinases are of papain character. However, Read and Haas (1937) express doubt as to the correctness of these views because they interpreted their extraction tests with bromate as opposed to the

[1] U. S. Patent Application Serial No. 36039, filed Aug. 13, 1935. Corresponding New Zealand Patent Application No. 74836, filed Aug. 26, 1935, and open for public inspection from April 29, 1936.

proteinase inhibition theory—an interpretation which the author considers to be incorrect in the light of the additional experimental evidence presented as to the stimulating effect of yeast on the flour proteinases and also because of the differences in relative concentration of bromate in the aqueous suspensions and actual doughs.

There is good reason to class the wheat proteinases in the papain group. Falk and Winslow's work (1918), cited by Read and Haas, indicates that enzymes of the trypsin group are of little importance in baking-strength problems. These investigators studied the influence of potassium bromate on Merck's, Fairchild Brothers', and Foster's "Trypsin" and on Merck's "Pancreatin" (a trypsin preparation) when acting on casein as a substrate. They found that, particularly in low concentrations (1 part $KBrO_3$ per 100,000 parts liquid), the activity of trypsin was increased by the bromate, whereas the activity of pancreatin was slightly inhibited at a bromate concentration of 1 : 10,000 and stimulated in lower concentrations (1 : 200,000). Since "Pancreatin" is also a trypsin preparation, it is curious that the results differ; however, careful examination of the work of Falk and Winslow indicates that their experimental technique did not permit of good agreement between parallel tests and it is felt that their conclusions on the relative effect of bromate on trypsin and pancreatin activity are hardly justified. If these workers had included papain in their studies, the strong inhibitory action of bromates on its activity would have been established and the fundamental nature of bromate action in relation to baking would have been cleared up many years ago. That the inhibiting action of bromate on papain, when acting on casein as a substrate, is very strong is shown in Table V, reproduced from a recent paper by the author (1938).

TABLE V

DIGESTION OF CASEIN BY PAPAIN IN PRESENCE OF VARIOUS HALOGEN SALTS [1]

Halogen salt	Nitrogen rendered soluble per 20 c.c. extract
	mg.
KCl	16
KBr	16
KI	16
$KClO_3$	16
$KBrO_3$	3.9
KIO_3	3.5

[1] In these tests, 4 g. casein and 0.08 g. Witte's papain were extracted with 50 c.c. acetate buffer (pH 4.62) plus 50 c.c. 0.004 M solution of the specified halogen salts for 2 hours at 35° C.

Summary

The author's theory that potassium bromate and ascorbic acid act as baking-strength improvers through inhibition of the flour proteinases is summarized and the work of Read and Haas, who interpret their experimental data as opposed to this theory, critically examined.

It has been pointed out that, in interpreting the results of their extraction tests, these workers did not take into consideration the stimulating effect of yeast on the flour proteinases or the fact that larger quantities of potassium bromate would be required in extraction tests than in doughs to produce a corresponding reduction in nitrogen solubility because of the much wider flour-water ratio. Experiments are reported which show that, when due consideration is given to these points, dosages of bromate, corresponding to those used in baking, reduce the solubility of the flour nitrogen.

It is also proved that the increase in water-soluble nitrogen reported by Read and Haas through the use of an excessive amount of ascorbic acid, is due to a reduction in pH; when this pH effect is eliminated, ascorbic acid reduces nitrogen solubility in all dosages employed. The author's theory is thereby confirmed.

Evidence strongly points to the wheat proteinases as belonging to the papain group of proteinases. Potassium bromate and iodate strongly inhibit the activity of papain on casein.

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STUDIES ON THE BAKING QUALITY OF FLOUR AS AFFECTED BY CERTAIN ENZYME ACTIONS. VI. FURTHER STUDIES RELATING TO THE ACTIVATION AND INHIBITION OF FLOUR PROTEINASE [1]

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(Read at the Annual Meeting, May 1938)

In previous papers dealing with the baking quality of flour as influenced by certain enzyme actions, we have considered the following angles of the problem: (1) the beneficial effects of proteolytic activity on the baking behavior of flours which develop "bucky" doughs (1934); (2) the separation of proteinase from the amylases; baking tests designed to measure the effects of these enzymic factors on "bucky" doughs, and the liquefying power of diastatic agents (1936); (3) purification of amylase, and the relative potency of the several enzymic fractions (1937); and (4), (5) the response of some proteinases to oxidizing and reducing agents with reference to activation and inactivation (1937 and 1938).

The results to be recorded in the present paper are essentially an elaboration of the two latter studies, conducted for the purpose of securing additional experimental information relative to the activation and inactivation of the proteinase contained in flour and malt products.

[1] Subsequent to the preparation of this manuscript for the Cincinnati convention of the American Association of Cereal Chemists, H. Jørgensen (*in absentia*) offered a paper at the same meeting in which he took issue with some previously published findings of Read and Haas. Although the latter have not yet had an opportunity to read Jørgensen's paper in full, they consider that the results here presented constitute further evidence in support of their views as previously published.

Brief Review of Literature

Mueller (1936) reported valuable studies concerned with the evaluation of dough quality by physical means. Experiments designed to show the effect of chemical treatment on the aging and extensibility of flour doughs failed to support Jørgensen's theory, that the benefits derived from bromate result entirely from proteinase inhibition. Physical studies of this particular nature, apparently definitely related to the bromate problem, give rise to questions other than the concept of inhibition.

In a paper concerned with the baking quality of flour doughs in relation to softening of the dough with age, Halton (1938) has pointed out that this phenomenon may be attributed to syneresis just as satisfactorily as it may be credited to enzyme action, as stated by Jørgensen (1936).

Sullivan, Howe, and Schmalz (1936) have cautioned against too narrow a view regarding the activation of proteolytic enzymes by substances containing the sulfhydryl group, because many compounds, inorganic as well as organic, can materially influence the colloidal behavior of the gluten proteins by modifying the oxidation-reduction system.

Hills and Bailey (1938) found that papain digestion increased the β-amylase activity of ungerminated barley approximately 100%, due to proteolytic release of β-amylase associated with water-soluble material.

Elion and Elion (1932) patented the process of increasing the saccharogenic (β-amylase) activity of flour appreciably by applying persulphate of potassium or ammonium in amounts varying from 0.25 to 0.02% by weight of flour. This is interesting in connection with the results of Hills and Bailey just cited.

Swanson (1937), using the recording dough mixer, evaluated graphically the condition produced in doughs by several treatments. Certain of Swanson's findings are at variance with those of Jørgensen (1936), namely the presence of significant quantities of free activating factors in a supercentrifuged aqueous extract of macerated yeast. The proteinase activity of malt wheat flour, if of any significance, was too slight to be detected, but the malt flour acted to make the dough more pliable. Proteinase activity (pepsin) was not inhibited by bromate.

Freilich and Frey (1937) reported that the incorporation of oxygen into bread doughs inhibited the effect of papain, although indirectly, since the inhibition is directly proportional to the amount of flour treated with oxygen. Treating papain alone with oxygen did not reduce its potency.

In connection with studies dealing with the action of ascorbic acid as a bread improver, Melville and Shattock (1938) have stated that dehydro-ascorbic acid is equivalent to bromate on a weight-for-weight basis, and is about four times more potent than is ascorbic acid on flours requiring 0.004% of bromate, an excessive dosage for flours in

Fig. 1. Baking results with flour No. 167880, showing effect on crumb structure and loaf volume produced by suitable amounts of glutathione, as compared to control (no treatment) and a 0.35-g. application of Merck's diastase of malt (U. S. P. IX), a medicinal preparation which is highly proteolytic. Additions were made to the sponge.

general. On the basis of data secured, they hold to the opinion that ascorbic acid *per se* is inactive in relation to such flours, and state that flour contains a mechanism whereby the oxidation of ascorbic acid to dehydro-ascorbic is brought about by an enzyme commonly called "ascorbic acid oxidase," a factor which would probably exist in variable amounts in flours, and thereby might explain the discrepancies

which appeared in some of their experiments conducted on a range of flours.

Hopkins and Morgan (1936) made a quantitative investigation of the system glutathione—ascorbic acid—enzyme. They found that so long as glutathione remained in the system it completely prevented

Fig. 2. Baking tests with flour No. 167880, showing effect on crumb structure and loaf volume produced by suitable quantities of glutathione and cysteine when added to the sponge.

the oxidation of ascorbic acid, but was itself oxidized. Only when glutathione had practically disappeared from the system did the oxidation of ascorbic acid begin. Glutathione also completely protected ascorbic acid from oxidation by copper catalysis.

Stotz, Carter, and King (1937) have criticized the use of "ascorbic acid oxidase," "vitamin C oxidase," and "hexuronic acid oxidase," as

interchangeable terms for a supposedly enzymic factor which acts in a specific manner to catalyze the aerobic oxidation of vitamin C. They suggest that some copper complex may perform the catalytic role.

Halton and Scott Blair (1937) have considered certain physical properties of flour doughs in relation to their bread-making qualities.

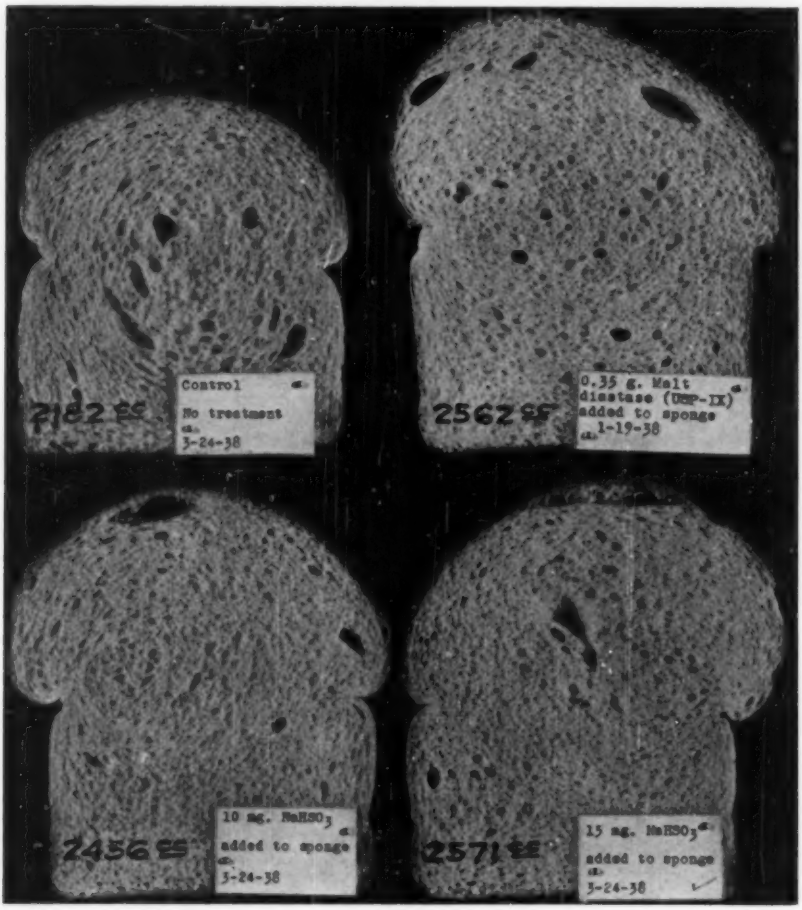

Fig. 3. Baking results with flour No. 167880, showing effect on crumb structure and loaf volume produced by suitable quantities of sodium bisulphite when added to the sponge. Compare to control; also the customary dosage of Merck's diastase of malt—U. S. P. IX, which we have applied to flours giving rise to "bucky" doughs. This product is highly proteolytic.

They have pictured, rather aptly, the mechanism of dough behavior during fermentation, wherein the gluten of flour dough consists of protein chains, which, acting like coiled springs, are responsible for the elastic behavior of the dough. Reference is made to this publication because it is probable that investigations of this type offer definite

angles of attack for determining the role (multiple or otherwise) played by bromate in baking processes.

Experimental

Flour.—The flour (No. 167880) used for the baking tests was milled from 50-lb. test wheat grown under adverse dry-weather conditions. Soon after cutting, excessive rainfall caused some sprouting in certain areas. Apparently, this accounts for its abnormally high saccharogenic activity of 642 (Blish-Sandstedt units), and a gassing power of 2036 c.c. (per 100 g. flour) as measured on the Werner Gasometer. The flour had been rather heavily Agene bleached, and whether its "bucky" character should be credited chiefly to overbleaching or to a combination of causes is uncertain.

Baking tests.—Using sponge doughs, numerous baking tests were made to determine the response of this flour to varying amounts of cysteine, glutathione, and sodium bisulphite when incorporated in the sponge. Results of these tests are illustrated in Figures 1, 2 and 3. Our interest in making these tests centered in the possible differences in the action of these agents on the gluten as determined by "feel" and "appearance" of the dough, and also the proper dosage requried to produce good loaves from a flour giving rise to "bucky" doughs.

Gelatin liquefaction.—As in previous studies, 25 c.c. of a 5% gelatin solution was used for evaluating the relative amount of liquefaction under specified conditions. The data contained in Tables I and II represent the conclusions reached from a study of a large number of tests, which, if tabulated separately, would require numerous tables. Relative viscosities, measured in terms of the time factor involved to deliver a fixed volume from a 100 c.c. specified pipette, gave similar results. It is realized that results obtained on a gelatin substrate might differ from those obtainable on gluten. A 5% solution of the gelatin used throughout our studies showed a pH range of from 5.4 to 5.7. The gelatin was an imported product always purchased from the same source. The pH of ordinary gelatins may vary widely.

Malt flour (wheat) XXII.—The wheat from which this flour was prepared in the laboratory was germinated for eight days at around 52° F. The average acrospire length was approximately 0.75 inch. After drying and removal of vegetative growth, the grain was ground and sieved. The malt flour, as used, consisted of the portion passing through a standard 80-mesh sieve. The saccharogenic index of this malt was 825, an unusually high index value. Ordinary malt flours generally range from 200 to 250. The proteolytic power of the specially prepared malt was correspondingly high, thereby making it

suitable material for gelatin studies. Commercial malt flours were not found to be very satisfactory because of their relatively weak proteolytic activity.

Sodium acid sulphite.—The incorporation of suitable quantities of sodium acid sulphite in the sponge mix produced essentially the same net results in conditioning the gluten as did glutathione and cysteine, but the behavior of this salt toward gluten appeared to be different. The "feel" and fibrous condition of the sponge was so dissimilar that it became immediately noticeable after a given period of fermentation. This salt has been referred to as an activator for papainases, but it will be observed from Table I that it greatly repressed the proteolytic activity of papain, bromelin and malt flour XXII.

Glutathione.—The glutathione was purchased from Eastman Kodak Co., but not prepared by them. We understand it is of German manufacture. Further information was unavailable except that it was considered to be essentially the reduced form.

TABLE I

RESPONSE OF CERTAIN PROTEASES TO REDUCING AGENTS

	Treatment applied			
Enzymic product	5 and 10 mg. glutathione	5 and 10 mg. cysteine	5 and 10 mg. vitamin C	5 and 10 mg. $NaHSO_3$
Merck's diastase U. S. P. IX [1]	Slight inhibition	Slight inhibition	Slight inhibition	Appreciable inhibition
Taka diastase	Slight inhibition	Slight inhibition	Slight inhibition	Appreciable inhibition
Papain	Marked activation	Marked activation	Marked inhibition	Marked inhibition
Bromelin (pineapple juice)	Marked activation	Marked activation	Marked inhibition	Marked inhibition
Trypsin	Slight inhibition	Slight inhibition	Slight inhibition	Slight inhibition
Powdered malt extract [1] (103° L.)	No activation	No activation	Appreciable inhibition	Without effect
Special malt flour (wheat) XXII	Marked activation	Marked activation	No inhibition	Marked inhibition
Malt syrup [1] (390° L.)	No activation	No activation	Slight inhibition	Slight inhibition

[1] It will be observed from this table that malt products which had been processed exhibited a different response from the wheat malt flour XXII.

TABLE II

RESPONSE OF CERTAIN PROTEASES TO OXIDANTS

Enzymic product	*Treatment applied*[1] 5 and 10 mg. $NaClO_2$	5 and 10 mg. $KBrO_3$	$KH(IO_3)_2$ KIO_4 $K_2S_2O_8$ (Dosage: 1 c.c. N/10)
Merck's diastase U. S. P. IX	Slight inhibition	Very slight inhibition	Very slight inhibition
Taka diastase	Slight inhibition	No inhibition	Very slight inhibition
Papain	Marked inhibition	Marked inhibition	Marked inhibition
Bromelin (pineapple juice)	Marked inhibition	Marked inhibition	Marked inhibition
Trypsin	Slight inhibition	No inhibition	No appreciable effect
Powdered malt extract (103° L.)	Appreciable inhibition	Very slight inhibition	Inhibitive action slight
Special malt flour (wheat) XXII	Appreciable inhibition	Appreciable inhibition	Measurable inhibition
Malt syrup (390° L.)	Appreciable inhibition	Measurable inhibition	Measurable inhibition

[1] The quantities of oxidants listed in this table are very excessive in relation to the dosages of enzymic product required. Proper enzymic controls were run simultaneously. With reference to the four malt products, no apparently significant repressive action was detected when the potassuim salts were applied in quantities suitable for baking practice.

Discussion

The data recorded in Tables I and II show the response which followed the application of certain oxidizing and reducing agents to eight enzymic products, three of which are utilized in commercial baking. All have proteolytic activity. It will be observed from Table I that reducing agents, such as glutathione, cysteine, vitamin C, and sodium bisulphite, exert a varied influence. Papain, bromelin, and malt flour were activated by the presence of glutathione, while Merck's diastase of malt, powdered malt extract, malt syrup, trypsin, and taka diastase, were either slightly inhibited or failed to respond. The same was true for cysteine which in common with glutathione contains the sulfhydryl group. The other two reducing agents, vitamin C and $NaHSO_3$ exerted in every case an inhibitory action of varying degree. These statements are based on results obtained with a gelatin substrate. It should be noted that processed malt products, such as powdered malt extract, malt syrup, and Merck's diastase of

malt (U.S.P. IX) responded differently from wheat malt flour in its normal state.

Table II shows the reaction of the same proteolytically active agents toward certain oxidants. Here again we find that the response of papain and bromelin is far more pronounced than is that of the several malt products, together with trypsin and taka diastase. It is recognized that an application of 5 or 10 mg. (per 25 c.c. gelatin solution) is a very excessive dosage when considered in relation to the quantity of enzymic agent. However, in obtaining the more complete information on the basis of gelatin liquefaction, not recorded in Tables I and II, the dosages of chemical agents were scaled downwards from 5 mg. to as little as 0.05 mg.—the smaller applications falling within the range of baking practice. We wish to call attention particularly to the fact that dosages within the range of baking practice produced no apparently significant inhibition on malt products adapted to baking, as measured by the technique we have employed. With reference to gelatin splitting, the proteinase of normal malt flour was found to be far less responsive to inhibition by oxidants than was true for either commercially prepared papain, or for bromelin as it exists in the raw juice of the pineapple.

TABLE III

EFFECT OF CERTAIN PRODUCTS ON THE pH OF GELATIN SOLUTION

Additions were made to 25 c.c. of a 5% gelatin solution.

Treatment	pH
Gelatin control	5.68
5 mg. vitamin C	5.62
10 mg. vitamin C	5.52
20 mg. vitamin C	5.25
10 mg. cysteine hydrochloride	5.35
10 mg. glutathione	5.36
10 mg. $NaHSO_3$	5.57
2 g. special malt syrup (390° L.)	5.52
4 g. dry malt	5.41
5 g. ordinary malt flour	5.55
2 g. wheat malt flour XXII	5.52

All pH determinations in this study were made by means of the glass electrode immediately after the additions of the different reagents.

In Table IV are recorded the results of some tests conducted with vitamin C for the purpose of comparing its behavior toward papain and malt flour XXII. These data are informative, but do not justify conclusions before subjecting certain points to further detailed study. It may be noted, however, that papain and malt flour responded differently under approximately identical conditions except for slight variations in pH.

TABLE IV

COMPARATIVE EFFECT OF VITAMIN C ON WHEAT MALT FLOUR NO. XXII AND PAPAIN

Quantity enzymic agent added per flask		Vitamin C applications shown in milligrams							Enzymic control	Gelatin control
		5	2	1	0.5	0.2	0.1	0.05		
Section A, papain (8% aqueous extract) Consistency of gelatin after 20 hours' digestion at 30° C.										
	30°	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid
0.3 c.c.	0°	Very firm	Fairly firm	Fairly mobile	Viscous liquid	Viscous liquid	Viscous liquid	Liquid	Liquid	Very firm
pH		5.34	5.40	5.44	5.48	5.51	5.51	5.53	5.53	5.58
Section B, wheat malt flour XXII Consistency of gelatin after 20 hours' digestion at 30° C.										
	30°	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid
2 grams	0°	Very viscous liquid	Very soft gel	Soft gel	Soft gel	Soft gel	Soft gel	Soft gel	Soft gel	Very firm
pH		5.48	5.51	5.52	5.53	5.53	5.54	5.55	5.55	5.60
Consistency of gelatin after 24 hours' digestion at 35° C.										
	30°	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid
2 grams	0°	Very viscous liquid	Very viscous liquid	Very viscous liquid	Very viscous liquid	Very viscous liquid	Very viscous liquid	Very viscous liquid	Very viscous liquid	Very firm
pH		5.38	5.43	5.46	5.47	5.48	5.48	5.50	5.52	5.56

Summary

With the incorporation of suitable quantities of glutathione, cysteine, and sodium acid sulphite in the sponge, baking tests with flour No. 167880 indicated that the nature of the action of the inorganic reagent, in conditioning the gluten, was unlike that of either glutathione or cysteine. Our tests on gelatin have shown that sodium acid sulphite strongly inhibited the proteolytic activity of papain, bromelin, and malt flour. Under similar conditions glutathione and cysteine functioned as potent activators.

Tests made on gelatin have shown that the proteinase of wheat malt is far less responsive to inhibition by bromate than are papain and bromelin. When applied in quantities applicable to commercial baking practice, no significant repression of wheat proteinase was apparent.

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SOYBEAN AMYLASE. I. THE CONCENTRATION AND CHARACTERIZATION OF SOYBEAN AMYLASE [1]

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Studies have been carried on in this laboratory on the preparation and properties of amylase concentrates from germinated wheat and rye by Naylor, Spencer, and House (1925) and Creighton and Naylor (1933), from germinated corn by Bulbrook (1928), and from germinated oats by Naylor and Dawson (1936). This paper reports the results of preliminary investigations dealing with the amylase of soybeans.

Orestano and Zummo (1930) report, after heat-treatment studies, that their results do not indicate the existence of two different amylases in soybeans, one causing liquefaction and the other saccharification of starch. Later studies by Artom and Orestano (1931) led to the equations for liquefaction and saccharification by soybean amylase. Orestano (1933) also reports that soybean amylase contains only one amylase, probably β-amylase. Results of investigations by Teller (1936) indicate the presence of two amylases in soybeans, but show definitely that soybean amylase is a better sugar-former than any other amylase studied with the exception of the enzyme of dry sweet potato. Bray (1935) offers the interesting suggestion that the amount of available amylase in soybeans does not appear to increase during germination, and that the enzyme is chiefly β-amylase.

Amylases are usually classified by Kuhn's (1925) mutarotation method, by methods involving measurement of the quantity of reducing sugars produced by the action of the enzyme on starch paste, or by methods based on the liquefaction of starch paste as measured by viscosimetric procedures. Dextrinogenic activity is commonly estimated by the Wohlgemuth iodine method. The terms α- and β-amylase are thus defined by the specific method used. Of the several methods Kuhn's is preferable, but it does not provide a means of measuring small amounts of one type of amylase in the presence of an excess of the

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other. As used in this paper the terms α- and β-amylase are defined as follows:

β-amylase (saccharogenic)—the enzyme which hydrolyzes starch to β-maltose, thus giving a positive mutarotation by Kuhn's method.

α-amylase (amyloclastic)—the enzyme which liquefies and dextrinizes starch paste to products which will not give the typical starch-iodine blue as measured by the modified Wohlgemuth method of Creighton and Naylor (1933).

Experimental

Preparation of enzyme suspension.—Two-tenths gram samples of soybean, ground to pass a 40-mesh sieve, were weighed directly into an agate mortar and ground as fine as possible. A small amount of water, redistilled and ice-cold, was added and grinding continued until a thick paste was obtained. This was gradually diluted to a thin liquid, transferred to a 100-c.c. volumetric flask, made to volume with cold water, shaken thoroughly at intervals for about 30 minutes, allowed to settle for a short time, and the supernatant liquid tested for activity. The flask containing the suspension was kept continually in an ice bath.

Preparation of substrate.—The substrate used in all determinations was a soluble starch prepared by the General Chemical Company. A quantity of the starch was obtained, mixed, and the moisture content determined. Sufficient starch, on the oven-dry basis, for a 2% suspension was mixed with cold water to form a paste, poured into boiling water, and boiled for 2 minutes. The starch dispersion was cooled, transferred to a volumetric flask, buffered at pH 4.7 with 0.2M Na_2HPO_4 and 0.2M NaH_2PO_4, and made to volume at 37°–40° C. with water.

Determination of reducing equivalent expressed as maltose.—The original Hagedorn and Jensen (1923) micro method for determining blood sugar as modified to the macro scale by Blish and Sandstedt (1933) and adapted to the determination of diastatic activity by Gore and Steele (1935) has been used throughout these investigations. Because the protein content of starch is very low, the protein precipitation was eliminated. Martin (1938) has found that by increasing the Na_2CO_3 content of the potassium ferricyanide reducing solution to 50 g. per liter, greater precision in the determination of maltose is obtained. This modification was incorporated into the following procedure.

In determining the saccharogenic power of soybeans, 50 c.c. of the substrate was measured into a 125-c.c. Erlenmeyer flask and placed in a thermostat maintained at 40° C. Sufficient time was allowed for the material to reach the temperature of the water bath. One c.c. of the enzyme suspension, as measured from an accurately calibrated one-c.c.

pipette, was added to the substrate, mixed thoroughly, and allowed to digest for exactly 30 minutes. To accurately control the time of digestion it was found advantageous to start the digestions at one-minute intervals. Just before removal of a 5-c.c. sample the pipette was filled with the digestion mixture and was rapidly discharged back into the digestion flask by air pressure. This was repeated a second time. By so doing, no appreciable cooling of the digestion mixture would result when the final sample was taken. About 20 seconds before completion of digestion, the mixture was drawn into the pipette and the upper meniscus adjusted to the mark. Exactly at the end of 30 minutes the sample was delivered into 25 c.c. of the alkaline ferricyanide reducing solution. The 5-c.c. pipette used was so calibrated as to deliver 5 c.c. of water at 40° C. in 10 seconds. The samples were then heated in a boiling water bath for 15 minutes, cooled to 20° C., 25 c.c. of the acetic acid-$ZnSO_4$ reagent and 5 c.c. of 50% KI added, and titrated immediately with $Na_2S_2O_3$. Sufficient undigested starch remained in the digestion mixture to serve as the indicator.

The saccharogenic power of the soybean amylase concentrates was determined by a slight modification of the above procedure. With the amylase concentrates, 40 to 80 mg. of the dry material was suspended in the water by shaking in the volumetric flask rather than by grinding to a paste in an agate mortar. Five-tenths c.c. of the suspension so obtained was added to the substrate.

This method for determining the saccharogenic power has been found to be more precise and more easily adapted to general laboratory work than the usual methods for determining amylase activity.

Variation of amylase content during germination.—Because of a rapid development of molds, special precautions must be taken during germination. Removal of all broken or discolored beans, followed by fumigation with carbon disulphide according to the method described by Naylor and Dawson (1936) gave a bean which could be germinated satisfactorily in the light at 18°–20° C. without mold growth. The beans were spread in a single layer on moistened blotting paper in enameled pans and placed in a small glass hood to prevent excess dust contamination. Samples were removed at 24 hour intervals, crushed on a glass plate, dried for 12 hours in a stream of air maintained at 40–45° C., and stored in tightly stoppered bottles. Crushing the beans was essential to rapid and adequate drying. The dried samples were ground in a Wiley mill to pass a 40 mesh screen, and the saccharogenic power determined.

Three series of samples, each covering a six-day germination period, were tested. For comparative purposes the saccharogenic power (milligrams of maltose produced per milligram of air-dry bean) has been

calculated. These results are presented in Figure 1. It is evident from the curve of the average values that the available amylase in soybeans tends toward a slight decrease as germination proceeds. The marked increase of available amylase in other seeds during germination is shown by the data of Naylor and Dawson (1936). In their investigations the saccharogenic power varied from 5.1 in ungerminated oats to a maximum of 23.9 on the seventh day of germination. This compares with an average of 27.0 for ungerminated soybeans and an average of 25.2 on the sixth day of germination. Germination of soybeans was not carried further than six days because of the development of molds and the rapid decomposition of the beans beyond that period.

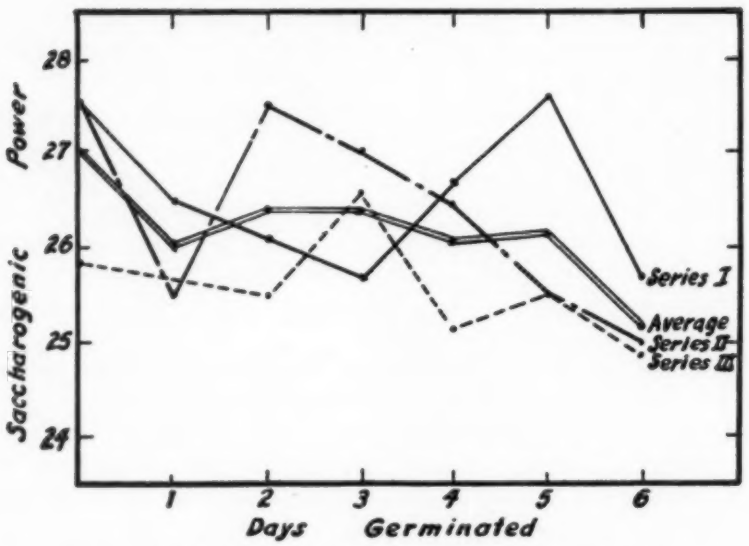

Fig. 1. Variation in saccharogenic power of soybeans during germination.

Preparation of amylase concentrates.—Water extraction of ground soybeans (40 mesh) followed by fractional precipitation with ethyl alcohol has shown that the greater portion of the enzyme is precipitated at an alcohol concentration of 65% by volume. Addition of alcohol to 85% yielded a concentrate only very slightly active.

Removal of these precipitates by filtration is a tedious and nearly impossible task. When they are thus obtained the precipitates are highly contaminated with filter paper. Only by adopting a technique based on centrifugation in a Sharples supercentrifuge has it been possible to obtain uncontaminated products in satisfactory yields. Further trouble was also caused by the oil-water emulsions formed when the soybeans were shaken with water during the extraction. These emul-

sions were entirely eliminated by first extracting the beans with ether in a modified Soxhlet extractor. Investigations of the amylase content of the soybeans before and after extraction have shown that the amylase present in the meal is only very slightly diminished during extraction with ether. Tests on the ether-free oil have failed to show any traces of amylase activity. After further studies of various phases of the preparation of the enzyme concentrate, the following procedure was adopted.

Good-quality, cured soybeans were ground in a Wiley mill to pass a 40-mesh screen. Two kilo lots of the ground beans were extracted about 20 times with diethyl ether in a modified Soxhlet extractor. The excess ether was allowed to evaporate at room temperature from the residue after extraction. Five hundred grams of the ether-extracted beans were covered with two liters of cold 50% alcohol. The temperature was maintained at that of running tap water (about 15° C.). The material was shaken at intervals for about three hours and then filtered through a double layer of cheese cloth, removing as much liquid as possible from the solid residue. This rough filtration was followed by centrifugation in a Sharples supercentrifuge at 20,000 r.p.m. The clear centrifugate was then cooled to 0° C. in an ice bath, and sufficient ice-cold absolute alcohol was added slowly, with continuous stirring, to raise the alcohol concentration to 65% by volume. The preparation was allowed to stand with occasional stirring for 15 minutes. The precipitate was then removed by centrifugation at 20,000 r.p.m., the precipitate collecting in the lower part of the bowl as a gelatinous, straw-colored film. This material was collected on a watch glass, dried under vacuum over $CaCl_2$, ground in an agate mortar, placed in a stoppered vial, and stored at a temperature of about 5° C. Based on the dry weight of the ether-extracted bean, yields of from 0.3 to 0.5% of enzyme concentrate were obtained. The saccharogenic power (milligrams maltose produced per milligram concentrate used) varied from 300 to 1360.

If in the foregoing procedure the alcohol concentration for precipitation is raised to 70% by volume, a much larger quantity of concentrate is obtained, but the saccharogenic power is almost quantitatively reduced. However, the concentrate so obtained is more soluble in water than the concentrate precipitated by 65% alcohol. For work other than concentration studies the more soluble preparation is of greater general use.

Although satisfactory yields of the amylase concentrate are obtained by the above method, only from 10 to 18% of the total amylase present in the beans is isolated as a solid concentrate. The task of improving this yield lies in future work.

Characterization of soybean amylase.—The enzyme concentrate prepared from soybeans was known to hydrolyze starch to products of high reducing value. To definitely establish the enzyme as an amylase, it was deemed necessary to identify maltose as a final degradation product. Fractionation of a typical digestion resulted in the isolation of a crystalline sugar and a dextrin-like material. This sugar was identified as maltose by its optical rotation and by the preparation and properties of two derivatives, the osazone and the octaacetate.

Following Kuhn's (1925) procedure, mutarotation studies of products formed in the early stages of hydrolysis of a substrate by soybean amylase show a positive mutarotation. The positive mutarotation shows an excess of β-maltose in the early stages of hydrolysis, and the enzyme is therefore classed as β-amylase. Giri's (1934) method of characterization also shows soybean amylase to be chiefly β-amylase.

Repeated determinations show that the 65% alcohol precipitates from soybean have a relatively high saccharogenic power. As compared with enzyme material prepared from rye and wheat by Naylor, Spencer, and House (1925) with a saccharogenic activity of 150 to 450, the enzyme concentrates prepared from soybean have saccharogenic powers of 300 to 1360. Application of Creighton and Naylor's (1933) modification of the Wohlgemuth method for the determination of amyloclastic power to representative samples of all fractions obtained in the concentration of soybean amylase gave values so low as to show approximately no α-amylase activity.

Discussion

Early investigations of soybean amylase point toward its identity as β-amylase. Teller's more recent data indicate that the starch-splitting enzyme of soybeans contains both α- and β-amylase. The work in this laboratory lends support to its characterization as β-amylase, with only a possible slight trace of α-amylase, both before and after germination. The only evidence that points toward the presence of α-amylase in soybean amylase is the marked ability of the concentrate to reduce the viscosity of starch paste. However, when we consider that the enzyme is one of the most powerful sugar-forming plant diastases, this sugar being formed by the hydrolysis of starch, it is not disturbing to observe a marked change in viscosity of the substrate during digestion with soybean amylase. More information as to the action of this enzyme on starch substrates is being furnished by work now in progress in this laboratory.

Such a convenient source of β-amylase concentrates in appreciable quantities as is furnished by the soybean is of considerable value in furnishing a tool for the characterization of starches and the degrada-

tion products derived therefrom. Martin (1938) and Martin and Newton (1938) have employed soybean amylase in such studies.

If concentration methods can be improved to insure better yields of the enzyme it is possible that soybeans may be used as a source of amylase preparations. Because of a decrease in amylase content during germination of the beans, germination will necessarily be eliminated from the preparative procedures. No special technical difficulties would be involved in preparing the concentrates, as concentration is a simple procedure when centrifugation is employed instead of filtration.

Summary

Methods are given for determination of the saccharogenic power of soybeans and soybean-amylase concentrates. These methods are adaptable to the same determination in other seeds.

The amylase content of soybeans decreases slightly during germination.

A method for preparing amylase concentrates from soybeans is given.

Characterization by various methods indicates that the concentrates contain principally β-amylase.

Acknowledgment

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A STUDY OF GLUTEN PROTEIN FRACTIONATION FROM SODIUM SALICYLATE SOLUTION. PART III. THE EFFECT OF PROTEOLYTIC ENZYMES

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In a recent publication the present writer (1938b) presented the results of a study of the effects of proteoclastic action upon wheat glutens washed from doughs and dispersed in sodium salicylate solution. Marked differences in the quantity of gluten dispersed in the early period of exposure to sodium salicylate, following the incorporation of proteolytic enzymes in the dough mix, were demonstrated. At that time I presented a review of the more pertinent literature dealing with proteolytic action in flour doughs and suspensions, and in view of this fact, as well as in consideration of the comprehensive lists of citations which have been published from time to time, I will not review the literature to any extent in the present article. The application of gluten protein fractionation from sodium salicylate dispersions by successive additions of $MgSO_4$ to the determination of gluten quality has been published (Harris, 1937, 1938a). This method has the advantage of rendering possible the recovery of the dispersed gluten in an apparently undenatured, unchanged condition, in contrast to the denaturing action of the classical solvents, dilute acid and alkali.

Experimental Material and Methods

A hard red spring wheat flour commercially milled from 1936 crop wheat was used in this investigation. This flour was a bleached first patent containing 13.1% protein and 0.40% ash, and had already been employed in determining the effect of treatments of proteolytic enzymes upon the solubility in sodium salicylate of the gluten washed from dough containing the customary dough ingredients plus enzyme and prepared in the usual manner. The procedure followed in the present instance was essentially the method outlined in the study of protein solubility, and consisted in washing the gluten from doughs prepared from 50 grams of flour plus dough ingredients, under a small standardized stream of 0.1% sodium phosphate solution (Dill and Alsberg, 1924). The concentration of gluten dispersed in 10% sodium salicylate was gradually stepped up until a concentration of 6 g. per 100 cc. was reached. This concentration was afterward used in this work. The procedure now followed was to place 12 grams of a finely divided gluten in 200 cc. of 10% sodium salicylate solution contained in a 250 or 300 cc. Erlenmeyer flask, and disperse with frequent shaking. Several days were allowed for the dispersion process to take place.

When dispersal appeared to have reached an end the dispersions were centrifuged, aliquoted, and 25 cc. portions were placed in 50 cc. centrifuge tubes and the protein progressively fractionated by the successive additions of 1.5, 4, and 10 cc. of concentrated $MgSO_4$ solution. Each fraction was removed by centrifuging from the residual liquid, washed with sodium salicylate plus $MgSO_4$ of the same concentration as the liquid from which the fraction had been precipitated, and transferred to Kjeldahl flasks for the determination of the quantity of protein contained in the fraction. All determinations of these fractions were run in triplicate. The quantity of wet crude gluten washed from each dough was also ascertained, as well as the moisture content of the gluten and the percentage of dry gluten. Drying was done in an electric oven at 105° C. for 48 hours. A large quantity of $MgSO_4$ solution was used in this study as compared with the quantity used in former work in order to remove as large a portion of the protein left in solution as possible. Even this increased addition did not completely clear up the solution when substantial quantities of certain proteoclastic enzymes had been added to the dough mix.

Discussion

The data obtained from a preliminary investigation of the effects of proteolytic enzymes upon gluten fractionation are shown in Table I. Papain was the enzyme incorporated in the dough mix in this instance.

A concentration of 4 grams of gluten to 200 cc. of 10% sodium salicylate solution was used. The addition of papain apparently increased the moisture content of the washed gluten, but had no definite effect upon the percentage of dry gluten itself. No marked trend was evident in respect to total quantity of gluten soluble in the dispersions and enzyme concentration in the dough. A decrease of gluten removed as fraction I is shown with increasing papain dosages. Fraction II tends to increase slightly, while fraction III shows a decided increase with greater proteolytic activity in the dough.

TABLE I

THE EFFECT OF ADDITIONS OF PAPAIN UPON THE RELATIVE DISTRIBUTION OF GLUTEN PROTEINS FRACTIONATED FROM SODIUM SALICYLATE SOLUTIONS

Treatment	Moisture	Wet crude gluten	Dry gluten	Solubility	Fraction I	Fraction II	Fraction III	Total protein fractions	Percent of total soluble
	%	%	%	*mgs.*	*mgs.*	*mgs.*	*mgs.*	*mgs.*	
Control	68.8	42.9	13.4	461	137	209	40	386	83.7
Papain, 0.003%	69.2	42.2	13.9	499	115	232	54	401	83.7
Papain, 0.006%	69.8	42.9	13.0	493	102	244	56	402	81.5
Papain, 0.010%	70.6	44.8	13.2	461	74	236	70	380	82.4

Concentration of gluten 4 grams per 200 cc. of dispersion.
Solubility and protein fractions are computed for Tables I, II, and III on the basis of 100 cc. of dispersion. Fractions are the quantity of protein removed by 6, 16, and 40 cc. of concentrated $MgSO_4$ solution, respectively.

In Table II the data obtained with papain as the added proteolytic agent revealed a very similar situation when a gluten concentration of 3 grams to 200 cc. of dispersion was used. A marked increase in fraction II is shown with the papain treatments. The decrease in fraction I is quite evident, as well as a corresponding increase in fraction III. A higher percentage of total protein appears to be removed from dispersion when the concentration of the dissolved gluten is increased.

TABLE II

THE EFFECT OF ADDITIONS OF PAPAIN UPON THE RELATIVE DISTRIBUTION OF GLUTEN PROTEINS FRACTIONATED FROM SODIUM SALICYLATE SOLUTIONS

Treatment	Moisture	Wet crude gluten	Dry gluten	Solubility	Fraction I	Fraction II	Fraction III	Total protein fractions	Percent of total soluble
	%	%	%	*mgs.*	*mgs.*	*mgs.*	*mgs.*	*mgs.*	
Control	67.4	38.0	12.4	855	286	413	58	757	88.5
Papain, 0.006%	69.8	40.4	12.3	992	264	535	74	873	94.7
Papain, 0.012%	70.4	47.3	14.0	940	187	527	79	793	84.4

Concentration of gluten 8 grams per 200 cc. of dispersion.

In Table III more comprehensive data are shown relative to the effect of a number of proteolytic enzymes upon gluten protein fractionation. The concentration of gluten in the work reported in this table was raised to 12 grams per 200 cc. of dispersion. Examining the papain extraction data first, it is evident that the increase in the quantity of gluten dispersed did not materially alter the effect produced by the papain additions upon the relative gluten fractions. A general rise in moisture content of the wet crude gluten and a decrease in percentage of dry gluten is evident with the higher treatments of papain. There is also evidence of increased gluten solubility with higher enzyme concentrations, although this solubility appears to become less as the highest papain treatments were used. A marked trend toward reduction of the protein removed as fraction I is shown as the enzyme concentration is raised. The second fraction tends to increase, then de-

TABLE III

THE EFFECT OF PROTEOLYTIC ENZYMES UPON THE RELATIVE DISTRIBUTION OF GLUTEN PROTEINS FRACTIONATED FROM SODIUM SALICYLATE SOLUTIONS

Sample	Moisture	Wet crude gluten	Dry gluten	Solubility	Fraction I	Fraction II	Fraction III	Total protein fractions	Percent of total soluble
	%	%	%	*mgs.*	*mgs.*	*mgs.*	*mgs.*	*mgs.*	
Control	67.1	39.6	13.3	1245	415	610	69	1094	87.9
Papain, 0.002%	68.1	43.7	13.9	1318	383	704	69	1156	87.7
Papain, 0.004%	68.8	44.6	13.9	1342	293	709	133	1135	84.6
Papain, 0.006%	66.3	37.0	12.5	1533	255	924	105	1284	83.8
Papain, 0.010%	69.4	39.2	12.0	1536	319	857	150	1320	85.9
Papain, 0.020%	69.2	39.0	12.0	1447	173	739	435	1347	93.1
Papain, 0.040%	71.4	41.5	11.9	1456	84	483	240	807	55.4
Papain, 0.060%	—	—	—	1323	73	210	492	775	58.6
Yeast water, 10 cc.	70.7	45.1	13.2	1325	524	593	53	1170	88.3
Yeast water, 20 cc.	71.9	42.7	12.0	1368	535	628	60	1223	89.4
Yeast water, 30 cc.	71.1	41.8	12.1	1378	521	636	53	1210	87.8
Pepsin, 0.04%	69.0	43.4	13.5	1143	395	524	103	1022	89.4
Pepsin, 0.12%	69.9	43.6	13.1	1354	494	634	83	1211	89.4
Pepsin, 0.20%	70.6	45.4	13.4	1316	448	638	77	1163	88.4
Pepsin, 0.30%	71.9	45.9	13.0	1277	415	650	74	1139	89.2
Pepsin, 0.50%	71.2	47.8	13.8	1348	415	680	114	1209	89.7
Pepsin, 1.00%	71.0	39.3	11.4	1516	452	805	75	1332	87.9
Pancreatin, 0.01%	67.3	40.1	13.1	1436	470	775	64	1309	91.2
Pancreatin, 0.02%	65.5	39.2	13.5	1516	452	805	75	1332	87.9
Pancreatin, 0.03%	68.1	41.2	13.1	1414	394	775	103	1272	89.9
Pancreatin, 0.04%	67.2	38.4	12.6	1493	406	830	96	1332	89.2
Malt diastase, 0.5%	68.6	42.9	13.2	1379	274	768	139	1181	85.6
Malt diastase, 1.0%	69.7	44.2	13.4	1385	194	734	224	1152	82.9
Taka diastase, 0.5%	68.7	40.2	12.6	1442	150	636	107	893	61.9
Taka diastase, 1.0%	69.6	41.4	12.6	1553	52	472	294	818	52.7

Concentration of gluten 12 grams per 200 cc. of dispersion.

crease, while fraction III increases sharply with added enzymes. The total protein removed from dispersion increases at first and then falls sharply later.

A different picture is presented by a study of the yeast-water data. Here we are presumably dealing with a protease activator, and the enzyme involved is a natural flour protease, which has been considered by many workers to be akin in nature to papain. The effects of these proteases upon wet crude gluten moisture and dry gluten percentages are similar to the effects of papain, the moisture content increasing and dry gluten percentage decreasing as more activating agent is added, while the solubility of the washed gluten is also increased as compared with the control. A very marked difference, however, is evident when the values obtained for fraction I by the two enzyme actions are compared. No evidence of a fall in the quantity of protein precipitated is shown with yeast-water increase, but actually an increase when compared with the quantity removed in the control. Fractions II and III, as well as the total protein removed from the dispersion, show no great differences for the different treatments.

In the instance of pepsin the same trend is visible in moisture content, dry gluten percentage, and, to some extent, in gluten solubility. No definite trends appear to be established in the case of fractions I and III, but fraction II clearly increases with addition of enzyme. Thus pepsin differs in its effect upon the distribution of these gluten protein fractions from both papain and flour protease, as would be expected from its source as an animal enzyme, whereas the two previous enzymes are of plant origin.

Pancreatin shows the same effect upon moisture, dry gluten, and gluten solubility as the other enzymes already considered, but resembles pepsin in its action upon fraction I. Pancreatin is more effective on a percentage basis than pepsin, although it belongs to the same class of enzyme. This enzyme also increases the protein removed in fraction II as compared with the control.

When the diastases are considered, the same relationships between enzyme concentrations and moisture, dry gluten, and gluten solubility are shown. A decided decrease in fraction I was yielded by both enzymes, but the effect of the two enzymes varies when fraction II is considered. Malt diastase tends to increase fraction II, but taka diastase apparently reduces the quantity of protein removed at this point of the fractionation process when present in substantial quantities. There are also significantly lower quantities of protein removed from dispersion when the doughs treated with taka diastase are considered, as compared with the other proteolytic agents, with the exception of the two highest treatments of papain.

When Table IV, which contains the gluten and fractionation data arranged as percentages of the control dough, is examined, these conclusions are brought out even more clearly. The moisture content of wet gluten is raised in some instances to 107% of the control by enzyme treatments, while a decrease in dry gluten is evident in the majority of the cases. Increases in gluten solubility range as high as 125% of control and in further investigations of this nature it would seem advisable to insure a constant level of dispersed gluten concentration. Changes in the percentages shown in fraction I are especially striking, running in one instance as low as only 12% of the control without enzyme treatment. Glutathione-treated doughs show marked increases in this fraction, and differ from the other data in this respect. Papain and pancreatin appear to have the greatest effect in increasing the quantity of protein thrown down as fraction II, while yeast water and taka diastase have the least effect. Papain also greatly increases

TABLE IV

THE EFFECT OF PROTEOLYTIC ENZYMES UPON MOISTURE CONTENT AND WEIGHT OF THE WASHED GLUTEN, AND THE DISTRIBUTION OF THE GLUTEN PROTEINS

Data Calculated as Percentages of the Untreated Control

Sample	Moisture	Wet crude gluten	Dry gluten	Solubility	Fraction I	Fraction II	Fraction III	Total fractions
	%	%	%	%	%	%	%	%
Papain, 0.002%	101	110	104	106	92	115	100	106
Papain, 0.004%	102	113	104	108	71	116	193	104
Papain, 0.006%	99	93	94	123	61	151	152	117
Papain, 0.010%	103	99	90	123	77	140	217	121
Papain, 0.020%	103	98	90	116	42	121	630	123
Papain, 0.040%	106	105	89	117	20	79	348	74
Papain, 0.060%	—	—	—	106	18	34	713	71
Yeast water, 10 cc.	105	114	99	106	126	97	77	107
Yeast water, 20 cc.	107	108	90	110	129	103	87	112
Yeast water, 30 cc.	106	106	91	111	125	104	77	111
Pepsin, 0.04%	103	110	102	92	95	86	149	93
Pepsin, 0.12%	104	110	98	109	119	104	120	111
Pepsin, 0.20%	105	115	101	106	108	105	112	106
Pepsin, 0.30%	107	106	101	103	100	107	107	104
Pepsin, 0.50%	106	121	98	109	100	111	165	110
Pepsin, 1.00%	106	99	85	122	109	132	109	122
Pancreatin, 0.01%	100	99	98	115	113	127	93	120
Pancreatin, 0.02%	98	99	102	122	109	132	109	122
Pancreatin, 0.03%	101	104	98	114	95	127	149	116
Pancreatin, 0.04%	100	97	95	120	98	136	139	122
Malt diastase 0.5%	102	92	99	90	66	126	201	108
Malt diastase 1.0%	104	112	101	111	47	120	325	105
Taka diastase 0.5%	102	101	95	116	36	104	155	82
Taka diastase 1.0%	104	104	95	125	12	77	426	75

TABLE V

THE EFFECT OF PROTEOLYTIC ENZYMES UPON GLUTEN PROTEIN FRACTIONATION FRACTIONS CALCULATED AS PERCENTAGES OF SOLUBLE PROTEIN

Sample	Fractions			Total protein removed
	I	II	III	
	%	%	%	%
Control	33.3	49.0	5.5	87.9
Papain, 0.002%	29.1	53.4	5.2	87.7
Papain, 0.004%	21.8	52.8	9.9	84.6
Papain, 0.006%	16.6	60.3	6.8	83.8
Papain, 0.010%	20.7	55.8	9.8	86.3
Papain, 0.020%	12.0	51.0	30.1	93.1
Papain, 0.040%	5.8	33.2	16.5	55.4
Papain, 0.060%	5.5	15.9	37.2	58.6
Yeast water, 10 cc.	39.5	44.8	4.0	88.3
Yeast water, 20 cc.	39.1	45.9	4.4	89.4
Yeast water, 30 cc.	37.8	46.2	3.8	87.8
Pepsin, 0.04%	34.6	45.8	9.0	89.4
Pepsin, 0.12%	36.5	46.8	6.1	89.4
Pepsin, 0.20%	34.0	48.5	5.9	88.4
Pepsin, 0.30%	32.5	50.9	5.8	89.2
Pepsin, 0.50%	30.8	50.4	8.5	89.7
Pepsin, 1.00%	29.8	53.2	4.9	87.9
Pancreatin, 0.01%	32.7	54.0	4.5	91.2
Pancreatin, 0.02%	29.8	53.1	4.9	87.8
Pancreatin, 0.03%	27.9	54.8	7.3	90.0
Pancreatin, 0.04%	27.2	55.6	6.4	89.2
Malt diastase 0.5%	19.9	55.7	10.1	85.7
Malt diastase 1.0%	14.0	53.0	16.2	83.2
Taka diastase 0.5%	10.4	44.1	7.4	61.9
Taka diastase 1.0%	3.3	30.4	18.9	52.7

the protein removed as fraction III, differing very markedly from the other enzymes examined in this respect. Yeast water, on the other hand, depresses the quantity of protein removed at this point. It is possible that increased severity of treatment with some of the other enzymes investigated, especially the diastases, might have the same effect in raising the final fraction and would therefore correspond to the effect of papain. Papain was used throughout in small dosages as compared with the relatively large concentrations of diastase employed in this study.

The fractionation data were recalculated and expressed as percentages of protein found to be soluble in the various dispersions. These calculations were made to obviate the possible influence of high gluten concentrations on the fractionation results. These values are shown in Table V. The data in this table yield much the same picture as

was presented in the former table, and emphasize the decrease in fraction I with substantial treatment of papain, malt diastase, and taka diastase. Papain and taka diastase both affected the quantity of protein removed from solution.

The effect of various proteolytic agents incorporated in dough upon the properties of gluten washed from these doughs may be summarized as follows: Proteolytic enzymes in general tend to increase the moisture content of the washed crude gluten, to decrease the percentage of dry gluten recovered from a dough, and to increase the solubility of the gluten in 10% sodium salicylate solution. These enzymes differ greatly among themselves, however, in their respective effects upon the relative distribution of the protein fractions of the dispersed gluten. Papain, malt, and taka diastase have been shown to have a very striking effect in reducing the quantity of gluten protein contained in fraction I. The flour proteases, when activated by glutathione, tend to increase the quantity of protein removed in fraction I, while pepsin and pancreatin do not have much effect. Initial treatments of papain raise the protein content of fraction II, but heavier additions cause a decrease. Malt diastase increases this fraction, while taka diastase appears to assert a depressive action, especially when present in 1.0% concentration. Flour protease is without appreciable effect upon fraction II, pepsin tends to raise this fraction with heavier dosages, and pancreatin increases fraction II at all concentrations used.

Fraction III is greatly augmented by increasing concentrations of papain, and the same effect is to be observed for both diastases studied in this investigation. Flour proteases, when activated by glutathione, are the only enzymes which appear to show a reducing effect upon this fraction. The total protein removed from these dispersions is increased by proteolytic action, with the exception of the heavier dosages of papain, and by taka diastase. Flour protease appears to differ distinctly from papain in its effect upon gluten fractionation, and the results obtained in this study, as well as from work reported by the author regarding the inhibiting effect of $KBrO_3$, would not justify their being classified in the same group of proteolytic enzymes. This is in opposition to certain statements which have appeared in the literature but agrees with the findings of Read and Haas (1937a), who postulated no repressive action of $KBrO_3$ upon flour proteases, as opposed to marked inhibition of papain and bromelin.

It is probable that papain, malt diastase, and taka diastase, in the concentrations employed in this study, exert a dispersive effect upon the gluten protein complex, causing the formation of smaller particles of gluten protein in sodium salicylate dispersion. Consequently the

quantity of protein removed from fraction I, which supposedly consists of the larger particles or micelles present in the dispersion, is decreased. As the protein removed in fraction I falls, more protein is removed in fraction II, as a result of an increase in the number of particles fractionated here because of a breaking down of the larger-size or first-fraction micelles. As the proteolytic action is stepped up, however, further disruptions of the gluten complex occur, and fraction II is diminished in quantity. This effect may be carried through fraction III, causing a smaller proportion of protein to be precipitated here, and may even affect the total quantity of protein removed because of inability to precipitate out these smaller particles from the dispersion by addition of $MgSO_4$ and centrifuging. The use of yeast water appears to induce a coagulative effect upon the flour gluten, causing more protein to be precipitated in fraction I, with less protein removed in the third fraction. Pepsin did not greatly alter the relative distribution of the fractions; at least no definite trends are apparent. These facts correspond with the theory that pepsin acts chiefly upon peptone and peptides. Pancreatin appeared to change the distribution of protein slightly, having first a coagulative, then a slight dispersive effect.

It is extremely probable that a shift in the pH of a dough would cause appreciable changes in the effect of the enzymes included in this study. A complicating factor in setting up an investigation of the action of proteolytic enzymes upon dough protein at various pH levels is the effect of these changes upon the characteristics of the flour gluten itself. It is probably also true that a change in dough temperature would cause changes in the relative effects of these various enzymes upon the properties of the gluten protein.

The conclusions reached in this study are essentially in agreement with results reported by Read and Haas (1934, 1936), Bohn and Bailey (1937), and Rupp and Bailey (1937) when investigating the effect of proteolytic enzymes upon dough and protein characteristics. These workers agree upon the protein-attacking power of proteolytic enzymes as registered in their liquefying effect upon gelatin, as well as in decreasing dough resistance to mechanical stress. No definite evidence was adduced in the present research to indicate a coagulative effect upon the gluten proteins by small additions of papain, as suggested by Balls and Hale (1936), but it is possible that this effect was produced by other proteolytic enzymes studied.

It is probable that proteolytic enzymes in general exert a progressive degrading action upon the gluten complex in breaking it down to smaller portions. This effect is reflected in the relative quantities of

protein successively fractionated, with a shift toward the region of smaller particles. This general result of proteolytic action might suggest that gluten protein is composed of one individual complex which may be split into almost innumerable fractions, these fractions being greatly affected in their distribution by proteolytic action. Other evidence obtained previously by the author and already published, however, appeared to indicate the presence of three distinct gluten complexes which differ among themselves in physical and chemical properties. The result of enzymic action on such a system would be that a mixture of these principal components would be obtained in the three fractions with each individual mixture varying with the enzyme treatment used. Physical differences in corresponding fractions caused by these treatments were very noticeable in many instances, and lend support to the theory, inasmuch as these separations did not always appear to consist of one homogeneous substance.

Summary and Conclusions

A series of 50-gram doughs were mixed from a hard red spring wheat flour, including simple basic-formula ingredients plus various proteolytic enzymes. These doughs were mixed in the usual manner, and the gluten immediately washed therefrom. These glutens were then dispersed as completely as possible in 10% sodium salicylate, and the protein fractionated by successive additions of appropriate quantities of $MgSO_4$. The protein content of these fractions was determined, and the quantity contained in the different fractions computed. A consideration of the summarized data would appear to justify the following conclusions:

Proteolytic enzymes added to dough may profoundly affect the relative distribution of the gluten protein fractionated from the washed-gluten dispersion of such doughs.

Various types of proteolytic enzymes affect the protein distribution in different ways, and marked dissimilarities are shown between different enzymes.

Papain, malt diastase, and taka diastase show evidences of a disruptive action upon the gluten complex, causing the formation of smaller protein particles in the dispersion, with consequent shifts in the quantities of protein contained in the various fractions.

Flour proteases, when activated by glutathione in concentrations present in the yeast water used in this work, appear to coagulate rather than disperse the gluten complex. Pancreatin may have a similar effect when present in moderate amounts. No definite trends were established from a study of the data obtained by the use of pepsin.

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IMMEDIATE EFFECT OF CROSS POLLINATION ON THE CAROTENOID PIGMENTS IN THE ENDOSPERM OF MAIZE [1]

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From previous investigation on carotenoid pigments in the endosperm of maize, it was shown that among inbred lines striking variations in concentrations of total carotenoid pigments occur.[3] To investigate further the magnitude of these differences, a survey was made in 1937 with a larger series of inbred lines. A study was made also to determine the immediate effect on the endosperm of cross

[1] Contribution from the Division of Agronomy and Plant Genetics and the Department of Botany, University of Minnesota. Paper No. 1633 of the Journal Series. Minnesota Agricultural Experiment Station.

[2] Associate Professor of Agronomy and Plant Genetics and Assistant Professor of Botany, respectively.

[3] I. J. Johnson and Elmer S. Miller, Variation in carotenoid pigment concentration among inbred and crossbred strains of corn, Cereal Chem. **15**: 345–350, 1938.

pollination between groups of high and low carotenoid-pigment strains. From the analysis of carotenoid pigments in the leaf tissue of the lines used in this study, it was possible to determine the relationship between the concentration of these pigments in the endosperm to that in the leaf tissue.

Material and Methods

The analysis of carotenoid-pigment concentration in the endosperm and in the leaf tissue was made with a group of 35 inbred lines of dent corn used in the corn-improvement program. These inbred lines had been inbred for several generations and were relatively homozygous for visible morphological characters. The seed of the inbred lines used for analysis was produced by sib pollination. A large number of crosses had been made in 1937 among these lines which provided the opportunity to study the immediate effect of cross pollination with lines that differed widely in concentration of endosperm carotenoids.

The seed, containing from 7% to 9% moisture, was finely ground in a Wiley mill until the meal passed through a 300-mesh sieve. No significant heating effects were observed when thus ground. The meal was tempered for 30 minutes as aqueous paste, and the final extraction was made by repeated grinding with sand in a mortar. After two extractions with acetone, followed by three to five with diethyl ether, the extractions were combined and transferred to a separatory funnel containing 150 c.c. of water. After the acetone in the extract was removed by two additional extractions with water, the solutions were made up to volume in ether.

The determination of total carotenoid-pigment concentration was made spectrophotometrically as described by Miller.[4]

Experimental Results

Variations in carotenoid pigment between strains.—Among the 35 inbred lines studied, a range from 0.89 to 2.57 mg. % total carotenoid pigments was obtained. Duplicate samples were analyzed from 15 strains to determine the extent of variability and to obtain a measure of the magnitude of the standard error. From the analysis of variance given in Table I, the F value of 116.0 greatly exceeds the one-percent point, indicating that the strains differed significantly in percentage of total carotenoid pigments. The low standard error of the mean of 0.0419 mg. % indicates that the method employed in analysis is very accurate. On the basis of the commonly accepted odds of 19 : 1 for significance, strains differing by 0.118 mg. % total carotenoid represent significant differences.

[4] Elmer S. Miller, Photoelectric spectrophotometry applied to the quantitative analysis of carotenoid and chlorophyll pigments in ternary and quaternary systems, Cereal Chem. 15: 310–316, 1938.

TABLE I

ANALYSIS OF VARIANCE OF 15 DUPLICATE SAMPLES FOR PER CENT CAROTENOID PIGMENTS IN THE ENDOSPERM OF MAIZE

Variance due to	D.F.	Sum sq.	Mean sq.	S.D.	F.
Varieties	14	5.6960	0.4069	—	116.0[1]
Samples	1	0.0001	0.0001	—	—
Error	14	0.0491	0.003507	0.0592	
Total	29	5.7452	—	—	—

[1] Exceeds the 1% point in level of significance. S. E. mean = 0.0419.

In previous studies with a smaller number of strains, an attempt was made to determine the relationship between visible endosperm color and the percentage of carotenoid pigments. The 35 lines analyzed in this study were classified into three groups: dark, medium, and light yellow endosperm color. The average percentage of total carotenoid pigments for each of the three groups is given in Table II.

TABLE II

AVERAGE MG. PER CENT AND RANGE IN TOTAL CAROTENOID PIGMENTS IN THE ENDOSPERM OF 35 INBRED STRAINS CLASSIFIED AS DARK, MEDIUM, AND LIGHT YELLOW IN COLOR

Endosperm color	No. of strains	Range in mg. % total carotenoids	Average mg. % total carotenoids
Dark yellow	8	1.40–2.57	1.89±0.1317
Medium yellow	18	0.98–2.09	1.67±0.0651
Light yellow	9	0.89–1.91	1.47±0.1031

The difference between the means of the dark and medium yellow lines is 1.5 times the standard error, between the medium and light yellow lines 1.6 times the standard error, and between the dark and light yellow endosperm lines 2.5 times the standard error of a difference.

The differences in total carotenoids between the color classes are not large, although the difference between the mean of the dark-yellow and light-yellow group exceeds the 5% point in level of significance and would indicate that dark-yellow endosperm lines of corn are higher in carotenoid pigments than the light-yellow lines. The range in mg. % total carotenoids was relatively large within each of the three color classes, probably as a result of difficulties in making accurate endosperm color classifications because of other pigments such as "brown aleurone," often associated with yellow endosperm corn.

The results of this study would indicate the possibility of selecting inbred lines high in carotenoid pigments as a means of improving the feeding value of corn in respect to vitamin A.

Immediate effect of cross pollination on carotenoid pigments.— Among the 35 inbred lines analyzed, 10 were classified as high and 5

as low in carotenoid-pigment concentration. In respect to pigment content, crossed seed was available in the following combinations: high female × high male, high female × low male, low female × high male, and low female × low male.

These types of crosses provided an opportunity to study the immediate effect of cross pollination when high carotenoid lines were pollinated with high carotenoid male parents in contrast with pollinations with low carotenoid male parents. Similar effects of pollination could also be studied when low carotenoid female parents were pollinated by high and low male parents, respectively.

From the data of this study given in Table III, it is evident that the average percentage of total carotenoids in the endosperm of crossed seed is significantly influenced by the genes for carotenoid pigments contributed by the male gametes when the parents differ in total carotenoid pigment percentage.

TABLE III

IMMEDIATE EFFECT OF CROSS POLLINATION ON THE PERCENTAGE OF TOTAL CAROTENOID PIGMENTS IN THE ENDOSPERM

Parents crossed	No. crosses	Average mg. % total carotenoids			Increase or decrease over female parent	Odds for significance [1]
		Female parent sibbed	Male parent sibbed	Cross		
High female × high male	16	2.16	1.98	2.08	−0.08	9 : 1
High female × low male	11	2.13	1.14	1.79	−0.34	10,000 : 1
Low female × high male	7	1.30	1.98	1.58	0.28	600 : 1
Low female × low male	5	1.27	1.18	1.35	0.08	3 : 1

[1] Student's pairing method and tables of Z.

When high-carotenoid-pigment female parents were pollinated by high-carotenoid-pigment male parents, or when low-carotenoid-pigment females were pollinated by low male parents, the crossed seed was not significantly different from the female parent. However, when high-carotenoid-pigment female parents were pollinated by low-carotenoid male parents, the crossed seed was significantly lower in pigment concentration than that of the sibbed seed of the female parent. Similarly, the crossed seed from low-carotenoid-pigment females pollinated by high-carotenoid-pigment males was significantly higher in pigments than the female parent. Among the eleven crosses between high-carotenoid female parents and low-carotenoid male parents, the crossed seed was lower than the female parent in all cases and in each of the seven crosses between low females and high males, the crossed seed had a higher percentage of total carotenoid pigments than the female parent. These results

would suggest that carotenoid pigments in the endosperm are subject to the xenia effects often found in endosperm characters.

On the basis that two-thirds of the endosperm genotype for carotenoid pigments is contributed by the female gametes and one-third by the male gametes, a very close agreement was found between the calculated value for the carotenoid pigments in the crossed seed and the values actually obtained. In the first group of crosses (high female × high male), the calculated value was 2.10 mg. % total carotenoids and the actual value was 2.08 mg. %. Similarly, in the crosses between high female × low male, low female × high male, and low female × low male, the calculated and actual values were 1.80 and 1.79, 1.52 and 1.58, and 1.24 and 1.35 mg. % respectively.

In a study with 35 inbred lines of the relationship between the percentage of total carotenoids in the leaf tissue and in the endosperm, a correlation coefficient of 0.1418 was obtained. Since this correlation does not approach the 5% point in level of significance, in this group of material the percentage of carotenoids in the leaf tissue apparently has no significant relation to that found in the endosperm. From previous investigations, it was shown that the leaf tissue of white endosperm lines contains as much carotenoid pigments as those from yellow endosperm strains. Apparently, the factors responsible for the formation of carotenoid pigments in the leaf tissue are not associated with those for the development of these pigments in the endosperm tissue.

Summary

A method is described which permits the quantitative extraction of carotenoids from maize endosperm.

Of the 35 inbred lines studied, it was found that significant variations occur with reference to carotenoid-pigment concentration.

When inbred lines were classified into dark, medium, and light yellow in endosperm color, the difference in total carotenoid pigments between the groups dark and medium, and medium and light, were not significant; but only the dark and light groups were statistically different.

When intercrosses were made between high female × low male, and low female × high male, the crossed seed was significantly different from the female parent, indicating that carotenoid pigments are subjected to the usual xenia effects.

No significant correlation was observed between the concentration of carotenoid pigments in the endosperm of inbred lines and that in the leaf tissues—suggesting that the two are due to independent factors or physiological processes.

THE LEAVENING ACTION OF AIR INCLUDED IN CAKE BATTER

J. A. DUNN and J. R. WHITE

Lever Brothers Co., Cambridge, Mass.

(Read at the Annual Meeting, May 1938)

In a communication presented before the 1937 annual convention, the present authors (1937) discussed the relationship between creaming volume, batter volume, and cake volume of lean pound cakes containing no chemical leavening agents. It was shown that whereas the correlation between creaming volume and finished cake volume is positive (subject to rather wide variations), the correlation between batter volume and finished-cake volume is practically a straight-line function. Because lightness of batter is directly associated with finished-cake volume, it follows that the amount of air incorporated into the batter in one of several ways should have a direct bearing on the volume of the finished cakes.

Using the method described previously,[1] we measured the specific volumes (the reciprocal of the specific gravity) of 6 ingredients used in the pound-cake test. Table I shows the proportions of these 6 ingredients used in the pound-cake batter and the specific volume or measure of lightness for these same ingredients.

TABLE I

PROPORTIONS AND SPECIFIC VOLUMES OF INGREDIENTS USED IN POUND-CAKE BATTER

	Weight used	Specific volume
	g.	
Sugar	900	1.15
Shortening	450	1.09
Salt	27	1.23
Eggs	450	0.97
Milk	500	0.966
Flour	900	1.75

If each of the foregoing amounts of ingredients is multiplied by its specific volume, it is apparent that the total volume of one batch of pound-cake batter should be 4,053 c.c. Dividing this figure by the total weight of the batch (3,227 g.), one obtains a specific volume of approximately 1.25. Without any mixing, this would be the theoretical lightness of a pound-cake batter, *if none of the ingredients*

[1] J. A. Dunn and J. R. White, Factor control in cake baking, Cereal Chemistry 14: 783–801, 1937.

were soluble in or miscible with water. Actually, the salt and sugar dissolve almost completely in the milk, with consequent loss in volume. Moreover, the flour absorbs liquid with consequent volume change.

An investigation of the leavening action of the air included in pound-cake batters seemed likely to result in some interesting data from which it was hoped some fundamental conclusions might be drawn.

Experimental

A double batch of pound-cake batter was mixed, and a batter volume of 1.37 was obtained. Half of the finished batter was placed in a vacuum desiccator and a vacuum carefully applied intermittently, with care taken to avoid losing any of the batter through the vacuum system. After a short period of time, the batter ceased to give up any air, and by this time it had taken on an appearance somewhat like that of custard cream. Normal amounts of this evacuated batter and of the control batter were scaled and baked according to our standard procedure.

At the same time, another batch of pound-cake batter was made according to a special procedure designed to avoid the incorporation of air. This procedure was essentially as follows: All of the soluble ingredients were dissolved in the milk and eggs. The flour was placed in the mixing machine, the liquid was poured in, and the batter mixed at low speed with a dough hook. At the end of the total mixing time of 1½ minutes, melted shortening was added and mixing continued until the shortening was distributed. The specific volume of this specially mixed batter was 0.87. The specific volume of the control was 1.37. The specific volume of the pound-cake batter which had been evacuated was 0.80. Figure 1 shows the cakes which resulted.

The cake on the right represents the "absolute zero" for this pound-cake formula. The volume of the batter placed in the pan was 908 c.c., and the volume of the resulting baked cake was 902 c.c. In other words, this cake exhibited absolutely no leavening action. The cake in the center, although ruined from a practical point of view, did expand somewhat during baking. The volume of the batter before baking was 987 c.c.; thus the increase during baking was 430 c.c. It is evident, therefore, that we were unable to mix a cake batter that was entirely devoid of included air. In all probability air was introduced by the flour and sugar, and to a lesser degree by the milk and eggs.

Some months later, a large batch of pound-cake batter was mixed and three-fourths of this batter was evacuated as previously described. That portion of the batter which had not been evacuated was used as a control and was baked according to the standard procedure. A second portion which had been evacuated was scaled and baked ac-

cording to standard procedure. A third portion was placed in the mixer and mixing was continued at low speed until a maximum batter volume had been reached. The fourth (evacuated) portion was placed in the mixer and mixed at medium speed until maximum specific volume was reached. The specific volume of the normal batter was 1.31. The specific volume of the evacuated batter was 0.80. After the evacuated batter was mixed at low speed for 40 minutes, a specific volume of 1.26 was obtained. Further mixing did not increase the lightness of batter. The portion of evacuated batter that was mixed at medium speed reached a maximum batter volume of 1.28 in 30 minutes, after which subsequent mixing did not increase the lightness. A cold-water bath was used during this remixing, in order to prevent overheating of the batter. The resulting cakes are shown in Figure 2.

Although it was impossible to cream into an evacuated batter the same amount of air which the batter previously contained, we were able to approach the previous lightness more closely than had been expected. The cakes produced by the remixed batters showed distinct evidences of overdevelopment of the gluten. The crust and crumb were toughened somewhat and the cakes tended toward a rather bold contour. The cakes resulting from the batter remixed at medium speed also showed excessive holes.

A sample of shortening was evacuated in the same manner in which the pound-cake batters had been handled until all of the air had been exhausted. A standard pound cake was made from this evacuated shortening, with a sample of the original shortening as a control. A batter volume and cake volume somewhat lower than the standard were obtained, after which we then endeavored to make up for the lack of air in the shortening by increasing the creaming time by 20%. This extra creaming raised the batter volume until it closely approximated that of the control. The loaf volume was likewise raised, but did not quite approach the volume of the control.

Figure 3 shows the resulting cakes and it is apparent that the air introduced during the manufacture of shortening tends to improve the quality of cakes and to reduce the time necessary to mix the cake. It will be noted that the decrease in volume caused by removing the air from the shortening is chiefly accounted for by side shrinkage rather than by a decrease in the height of these cakes. Extra creaming straightened the sides and reduced the shrinkage.

Discussion

On the basis of the data obtained, it has been possible to calculate how much of the volume increase is produced by the thermal expansion of the air included in the batter. The batter temperature was 78° F.,

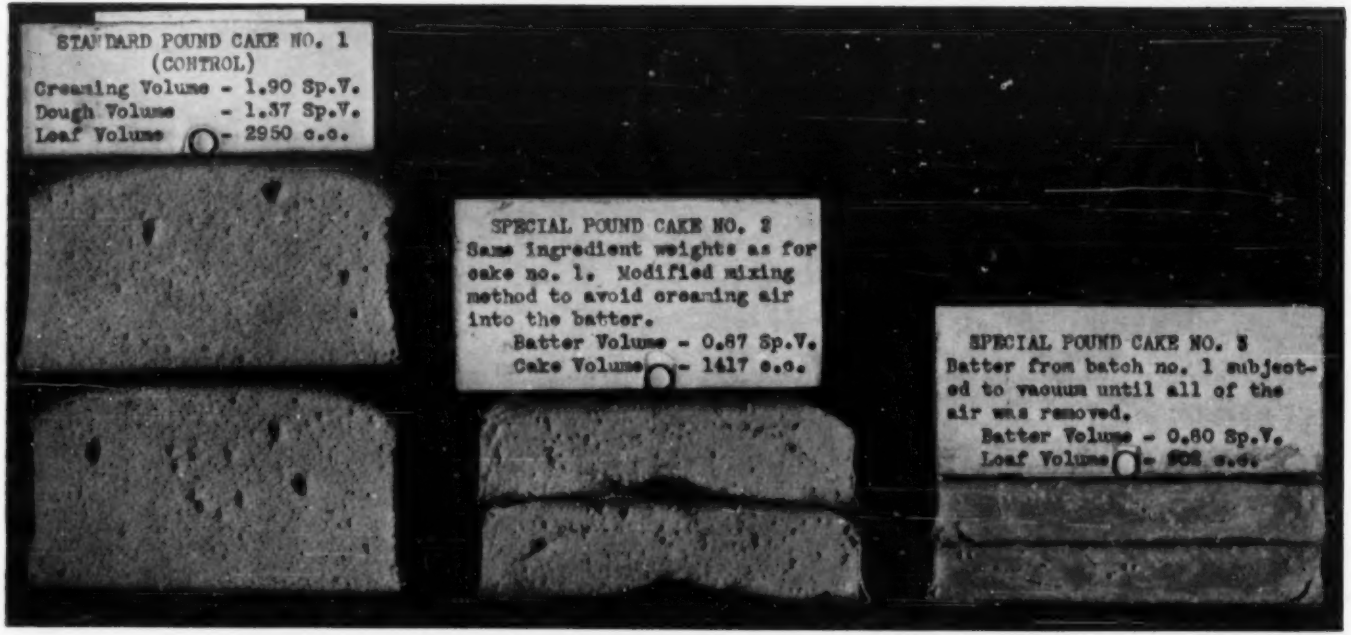

STANDARD POUND CAKE NO. 1
(CONTROL)
Creaming Volume - 1.90 Sp.V.
Dough Volume - 1.37 Sp.V.
Loaf Volume - 2950 c.c.
SPECIAL POUND CAKE NO. 2
Same ingredient weights as for cake no. 1. Modified mixing method to avoid creaming air into the batter.
Batter Volume - 0.87 Sp.V.
Cake Volume - 1417 c.c.
SPECIAL POUND CAKE NO. 3
Batter from batch no. 1 subjected to vacuum until all of the air was removed.
Batter Volume - 0.80 Sp.V.
Loaf Volume - 902 c.c.

Fig. 1. The leavening action of air included in pound-cake batter.

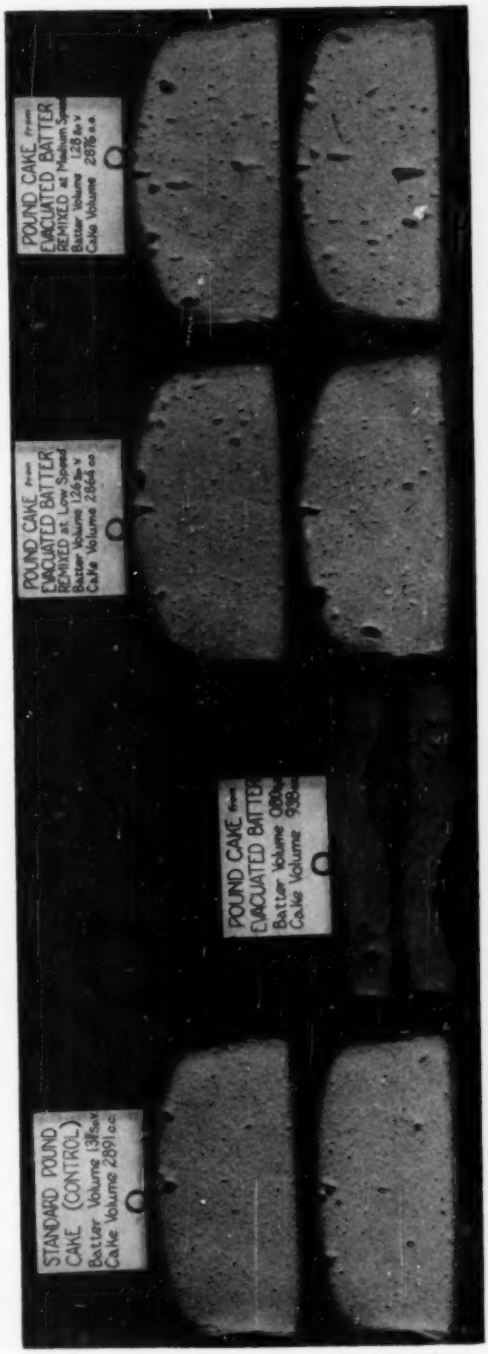

STANDARD POUND CAKE (CONTROL)
POUND CAKE from EVACUATED BATTER REMIXED at Low Speed
POUND CAKE from EVACUATED BATTER REMIXED at Medium Speed

Fig. 2. Air exhausted rom pound-cake batter may be replaced by extended mixing.

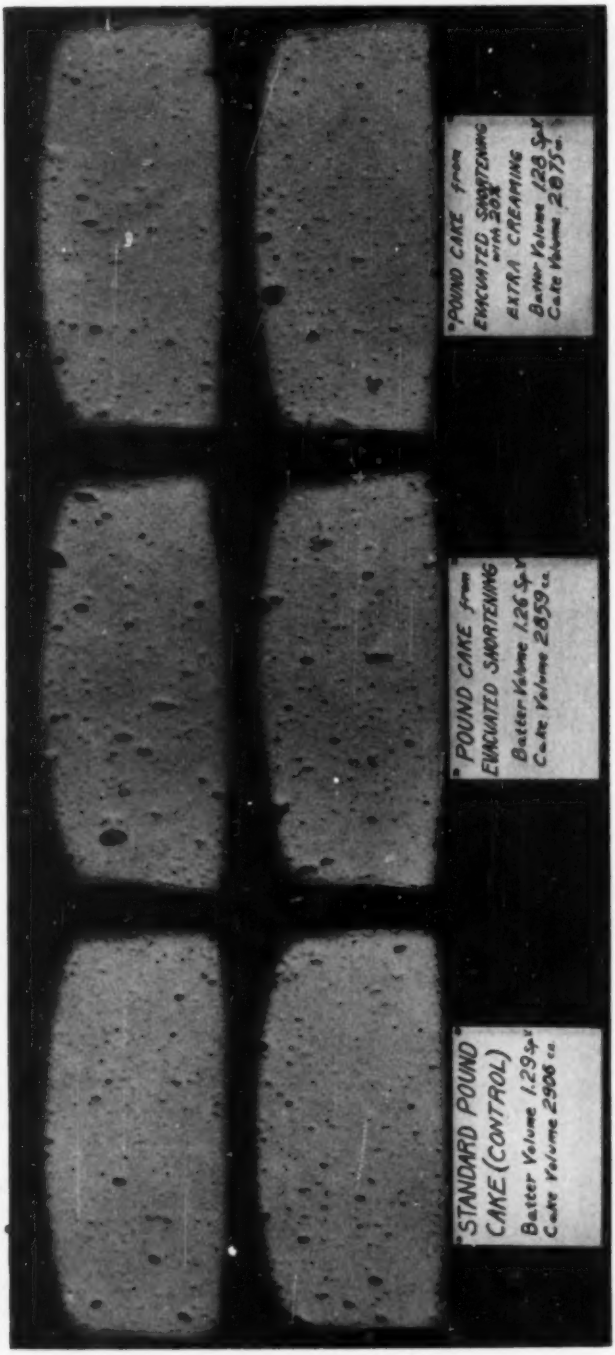

STANDARD POUND CAKE (CONTROL)
Batter Volume 1.29 qt
Cake Volume 2906 cc.
POUND CAKE from EVACUATED SHORTENING
Batter Volume 1.26 qt
Cake Volume 2859 cc.
POUND CAKE from EVACUATED SHORTENING WITH 20% EXTRA CREAMING
Batter Volume 1.28 qt
Cake Volume 2875 cc.

Fig. 3. Air introduced in the manufacture of shortening improves cakes and reduces creaming time.

which is 298.4° A. The temperature of the finished cake as it left the oven was 208° F., which is 370.8° A. Dividing the lower absolute temperature into the higher, therefore, we find the air expansion factor is 1.242.

From the scaling weight, which was 1,135 g., and the specific volume of the batter (1.37), it is possible to calculate the actual volume of the batter scaled in each pan (1,555 c.c.). The volume of the finished cake was measured (2,950 c.c.). The difference between these two values represents the true expansion or volume increase (1,395 c.c.).

Because the specific volume of the air-free batter has been determined, it is also easy to show that the total volume of the air-free batter is 908 c.c. Thus the batter scaled in each pan contained 647 c.c. of air. Applying our expansion factor of 1.242, we can account for 803.6 c.c. of the total volume increase, as directly caused by the thermal expansion of the air. This figure is equivalent to 51.7% of the total increase.

The remainder of the volume increase must be due to the evaporation of liquid into the tiny air cells, and the subsequent expansion of this water vapor. This portion of the oven-expansion (48.3%) will be subject, of course, to a correction factor because of possible changes in volume of starch and gluten due to the action of moisture and oven heat.

For all practical purposes, it is safe to conclude that half of the volume increase is due to the air incorporated in the batter, the remainder being principally produced by moisture evaporating into the air cells and subsequently expanding.

Applying the same method to the data procured by the special method designed to prevent mixing air into the batter, we obtained figures indicating that about a fourth of the increase in volume was attributable to the thermal expansion of the air, the remainder being caused principally by the production and expansion of water vapor. It is interesting to observe, however, that when all of the air is evacuated so that there are no air cells into which the moisture can evaporate, the volume increase is zero.

Summary

Half of the increase in volume of a lean pound cake containing no chemical leavening agents is due to the thermal expansion of air included in the batter.

When the air in a pound-cake batter is completely exhausted, a peculiar batter resembling custard cream is produced. When this batter is baked, the volume increase is zero.

It is possible to approach a batter containing no air by varying the mixing procedure; but even a small amount of air included in the batter results in a definite increase in volume during baking.

Air can be remixed into an evacuated batter and a reasonably good pound cake produced. The resulting cake, however, shows evidence of overmixing.

The incorporation of air in commercial shortenings improves the texture and volume of cake, and reduces the time required for proper creaming.

REPORT OF THE COMMITTEE ON TESTING RYE FLOUR

L. H. BAILEY, *Chairman*

Food Research Division, Bureau of Chemistry and Soils,
U. S. Department of Agriculture, Washington, D. C.

(Read at the Annual Meeting, May 1937)

Five years ago a committee was appointed by the American Association of Cereal Chemists to develop a method that could be used to test the baking qualities of rye flours. This committee pointed out in its first report that its task was a large one with many complications. There was no standard for rye bread and no universal conception as to the ideal loaf of rye. There are at least five grades of rye flours with variations within these grades. Varying percentages of rye flour are mixed with clear flour to make the rye bread. Sometimes a mixture of two grades of rye flour is used as the rye component. Rye breads, like white breads, are made with sponge and dough as well as with straight doughs. "Swedish" rye is a large loaf, open grained, and sweet to the taste. It is made with sugar or molasses while pumpernickel has a very close, compact grain, with a sour taste when made with "sour" dough.

Out of this maze of variations the committee decided to limit its study to straight doughs made with yeast, salt, water, clear flour and the five principal grades of rye flours. At first both pan and hearth loaves were made, but later the pan loaves were discontinued. Neither "Swedish" rye nor pumpernickel was included in the studies.

While the proportions of rye flours of the different grades and the clear flour varied in the experiments, the total flour constituent was always kept at 200 g. The doughs were mixed in Hobart-Swanson mixers, when available. The total fermentation time, the proofing time, when to punch the doughs, and how many punches to make were all given consideration.

After the bread was made the trouble was to score it. There was no score card for rye bread, so the committee proceeded to develop one. After much study it was concluded that one score card could be used

with all grades of rye bread, the only condition being that breads from the same grade of rye flours be scored against each other. Thus the white rye breads should be scored by themselves, the medium ryes by themselves, and so on. The committee has been using numerical values for the different points being considered in scoring. On this point there is divided opinion among the members of the committee, some considering it preferable to use descriptive terms rather than numbers to express variations. Loaf volume and weight are given as actual values, and it would be a simple matter to use descriptive terms instead of arbitrary numerical values for the other scores.

Under crumb color, the committee had great difficulty in reaching an agreement in scoring collaborative bakings. In scoring white rye breads perhaps the highest score should be given to the one having the whitest crumb, while in scoring extra dark ryes the highest score would perhaps be given to the one that had the darkest colored crumb, but when it came to the medium ryes the task was not so easy.

To overcome this difficulty a color panel was prepared from rye breads of six different shades of color, ranging from that of white rye bread to that of extra dark rye bread. These breads were made by the method as now used by the committee. The bread was sliced and dried, then impregnated with collodion and mounted on a pasteboard panel. It was a simple matter to place a slice of the bread being tested alongside these breads on the panel and note which one most nearly matched in color. If the color fell between two of the pieces on the panel the fact was indicated by the use of the + and − signs; for example, 2+ to indicate a color between 2 and 3 on the panel. The colors of the breads on the panel were believed to cover the range that is likely to be met when using formulas and methods outlined by the committee. The formulas and methods follow:

METHOD FOR TESTING RYE FLOURS

Formulas for using six grades of rye flour

White and light rye bread		Cream and medium rye bread		Dark and extra dark rye bread	
	Grams		*Grams*		*Grams*
Clear flour	100	Clear flour	120	Clear flour	140
White or light rye flour	100	Cream or medium rye flour	80	Dark or extra dark rye flour	60
Yeast	4	Yeast	4	Yeast	4
Salt	4	Salt	4	Salt	4
Water (variable)	(120 c.c.)?	Water (variable)	(130 c.c.)?	Water (variable)	(135 c.c.)?
Mix for 1 minute in Hobart-Swanson mixer or equivalent.		Mix for 1 minute in Hobart-Swanson mixer or equivalent.		Mix for 1 minute in Hobart-Swanson mixer or equivalent.	
First punch after 75 minutes		First punch after 75 minutes		First punch after 75 minutes	
Second punch after 45 minutes		Second punch after 45 minutes			
Mold after 30 minutes		Mold after 30 minutes		Mold after 45 minutes	
Proof to optimum		Proof to optimum		Proof to optimum	

After proofing, give three slight slashes across the top of the doughs and place in oven. Bake at 230° C. for 30 to 35 minutes. Use plenty of steam in the oven or brush the loaves with water two or three times after the crust has formed. Cool on rack and score (preferably the following day).

These methods are the result of several years' study. They have been tested by the committee and have proved satisfactory for the purpose of testing the baking qualities of rye flours.

The score card would be satisfactory to the majority of the committee if descriptive terms were substituted for numerical scores for general appearance, crust color, grain, texture, odor and taste.

Scoring crumb color by means of the color panel has been used only this last year. While the collaborative results do not show the uniformity that was anticipated, it is the best scheme that has been tried so far.

For the collaborative work this year the same samples of white, medium, and dark rye flours, together with a clear flour, were furnished each of the collaborators. Formulas and methods previously worked out by the committee were used by the collaborators in testing these flours. The scoring was done according to the score card developed by the committee, using numerical scores for certain of the points under consideration and the color panel (furnished each collaborator) for scoring crumb color. The collaborative results are shown on the score card given.

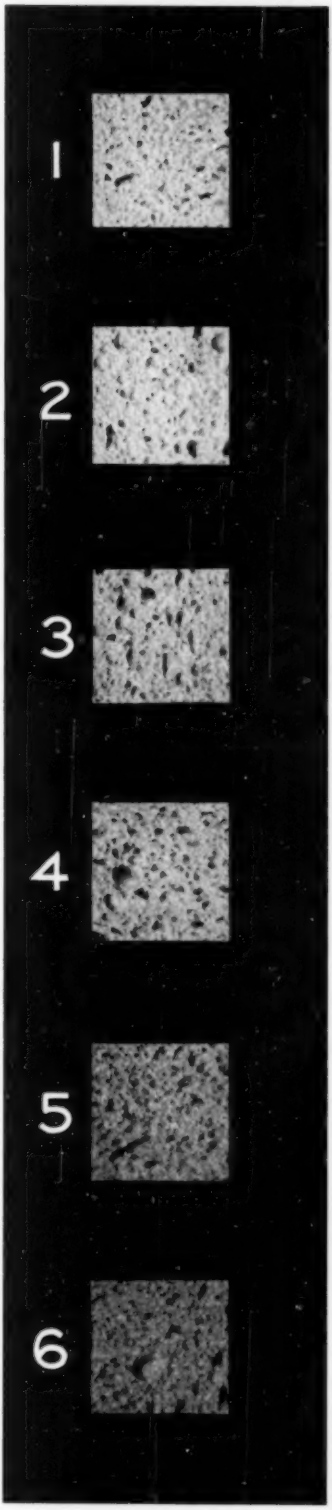
SCORE CARD FOR COLLABORATIVE BAKING TESTS OF RYE FLOURS

Collaborator	Rye flour grade	Loaf volume	Loaf weight	General appearance (25)	Crust color (10)	Crumb color (by panel)	Crumb grain (20)	Crumb texture (20)	Odor and taste (25)	Remarks
A	White	840	260	23	9.5	1+	19	19	25	Creamy
B	"	840	270	23	10.0	1	19	20	23	
C	"	618	252	25	9.0	1−	17	17	24	Creamy
D	"	756	265	23	9.0	1+	19	18	21	
E	"	770	274	23	9.0	1	18	18	25	
A	Medium	855	267	23	9.5	2−	19	19	25	
B	"	925	272	25	10.0	1	19	20	23	
C	"	562	259	25	10.0	2+	18	19	24	
D	"	808	275	24	9.0	3+	18	18	23	
E	"	850	277	24	9.0	2−	18	19	25	
A	Dark	790	292	23	9.0	5−	19	19	25	
B	"	805	267	23	10.0	3	20	20	23	
C	"	438	271	25	10.0	4	19	19	24	
D	"	725	295	21	8.0	4	17	17	24	
E	"	645	297	23	9.0	4+	17	16	25	

The collaborators were the following:

T. W. Sanford, Eagle Roller Mill Company, New Ulm, Minnesota.
G. Moen, General Mills, Inc., Minneapolis, Minnesota.
I. O. Juvrud, The W. E. Long Company, Chicago, Illinois.
J. T. Flohil, Pillsbury Flour Mills Company, Minneapolis, Minnesota.
L. H. Bailey, Bureau of Chemistry and Soils, U. S. Dept. of Agriculture, Washington, D. C.

RYE BREAD PANEL



This panel represents breads made with white, light, cream, medium, dark and extra dark rye flours in the proportions given in the methods proposed in this report.

Conclusions

The committee has now developed methods for testing rye flours of the different grades for their baking qualities.

It has developed a score card that is suitable for use with the different grades of rye flour. Descriptive terms may be substituted for numerical values if desired.

A color panel has been developed which shows variations in color between white rye bread and extra dark rye bread baked according to the methods prescribed.

It is recommended that:

The method here presented be adopted tentatively by this Association for the purpose of testing the baking qualities of rye flours.

(It is to be clearly understood that in developing this method no attempt has been made to standardize the commercial methods of making rye bread.)

It is further recommended that this committee, having completed its task, be discharged.

REPORT OF THE 1936–37 COMMITTEE ON CAKE BAKING TESTS AND SELF-RISING FLOURS

L. D. WHITING, *Chairman*

Ballard and Ballard Co., Inc., Louisville, Kentucky

(Read at the Annual Meeting, May 1937)

The committee on Cake Baking Tests, consisting of H. R. Fisher, J. W. Montzheimer, W. C. Rohrbaugh, W. E. Stokes, Laura K. Track, and L. D. Whiting, centered its attention this year on the comparison of the A. A. C. C. Basic Cake-Baking Method (designated as Method 1) with a yellow formula using whole egg (designated as Method 2). The latter method was based upon the formula used by Cooley and Davies (Cereal Chemistry **13**: 609–613, 1936).

Five soft-wheat flours varying widely in analysis, as shown in the following table, were baked by the five collaborators and scored according to the score card (Cereal Chemistry **8**: 253).

Flour	No. 1	No. 2	No. 3	No. 4	No. 5
Moisture, %	12.8	11.6	11.0	11.4	12.1
Protein,[1] %	8.2	7.6	9.4	7.1	7.6
Ash,[1] %	0.42	0.35	0.35	0.32	0.35
pH	5.54	5.27	5.6	5.65	5.1

[1] Moisture basis 13.5%.

The results are shown in Tables I to III and Tables IA to IIIA.

TABLE I
TOTAL AVERAGE SCORES

Collaborator	Flour no. 1 Method		Flour no. 2 Method		Flour no. 3 Method		Flour no. 4 Method		Flour no. 5 Method	
	1	2	1	2	1	2	1	2	1	2
A	61	76	68	75	67	80	45	64	53	75
B	57	48	77	78	73	70	59	54	86	96
C	82.3	83.5	87	90	84	84.5	77	78.5	89	91
D	65	56.5	87.5	89	80.25	67	71	65	97	94.5
E	79	75	87	85	75	71	72	69	89	90
Average	68.8	67.8	81.3	83.4	75.85	74.5	64.8	66.1	82.8	89.3

TABLE IA
COMPOSITE RATING
(By total average score)

	Method 1		Method 2	
	Score	Rating	Score	Rating
Flour 1	68.8	4	67.8	4
Flour 2	81.3	2	83.4	2
Flour 3	75.85	3	74.5	3
Flour 4	64.8	5	66.1	5
Flour 5	82.8	1	89.3	1

TABLE II
VOLUME SCORES

Collaborator	Flour no. 1 Method		Flour no. 2 Method		Flour no. 3 Method		Flour no. 4 Method		Flour no. 5 Method	
	1	2	1	2	1	2	1	2	1	2
A	10	12	10	12	10	15	6	11	8	12
B	8.5	7	13.5	12	15	12	8	6	15	15
C	11.75	12.5	13.5	14	14	12.5	12	12.5	13.5	14
D	10	12.5	16	15	15.75	9	13	11.5	20	15.5
E	13	12	14	13	12	11	10	12	14	14
Average	10.65	11.2	13.4	13.2	13.35	11.9	9.8	10.6	14.1	14.1

TABLE IIA
RATING BY AVERAGE VOLUME SCORE

	Method 1		Method 2	
	Score	Rating	Score	Rating
Flour 1	10.65	4	11.2	4
Flour 2	13.4	2	13.2	2
Flour 3	13.35	3	11.9	3
Flour 4	9.8	5	10.6	5
Flour 5	14.1	1	14.1	1

TABLE III

Total Average Internal Scores

Collaborator	Flour no. 1						Flour no. 2						Flour no. 3						Flour no. 4						Flour no. 5					
	Method 1			Method 2			Method 1			Method 2			Method 1			Method 2			Method 1			Method 2			Method 1			Method 2		
	Texture	Grain	Color	Texture	Grain	Color	Texture	Grain	Color	Texture	Grain	Color	Texture	Grain	Color	Texture	Grain	Color	Texture	Grain	Color	Texture	Grain	Color	Texture	Grain	Color	Texture	Grain	Color
A	16	15	10	22	20	10	20	16	12	23	18	12	20	15	8	25	20	8	16	10	5	21	18	5	16	10	11	22	18	11
B	14	13.5	7	11	8	10	21	16	12	20	20	12	20	14	11	20	14	11	14	15	8	13	15	8	21.5	21.5	14	28	24	14
C	23	21	13	26.5	21	11	26	21.5	13	28	21.5	13.5	23	24	12	28	21	11	22	20	10	23	21	10	26	22.5	14	28	22	14
D	18	15	10	13	10	12	23	22	12	26	22	14	21	18	11	18	15	14	20	16	10	20	12	13	29	20	14	30	22	15
E	24	18	12	20	20	12	26	22	13	25	22	13	22	19	11	19	19	12	22	17	11	19	18	12	26	22	14	28	22	13
Average	19	16.5	10.4	18.5	15.8	11	23.2	19.5	12.4	24.4	20.7	12.9	21.2	18	10.6	22	17.8	11.2	18.8	15.6	8.8	19.2	16.8	9.6	23.7	19.2	13.4	27.2	21.6	13.4
Av. total	45.9			45.3			55.1			58.0			49.8			51.0			43.2			45.6			56.3			62.2		

TABLE IIIA

Rating by Internal Score

	Method 1					Method 2				
	Texture	Grain	Color	Score	Rating	Texture	Grain	Color	Score	Rating
Flour 1	4	4	4	45.9	4	5	5	4	45.3	5
Flour 2	2	1	2	55.1	2	2	2	2	58.0	2
Flour 3	3	3	3	49.8	3	3	3	3	51.0	3
Flour 4	5	5	5	43.2	5	4	4	5	45.6	4
Flour 5	1	2	1	56.3	1	1	1	1	62.2	1

Summary

The total average score figures for cakes made by the two methods very closely approximated each other. The effect of method of mixing upon general scoring was very little in comparison with the differences brought out in the cakes by varying the flour.

The general effect of the total average score (Table I) for the series of cakes made by the five collaborators in this test was to rate the methods as the same. The flour ratings ranked the same for both Methods 1 and 2 (Table IA).

When the volume score (Table II) was viewed separately, the difference between methods was little but the differences from flour sample to flour sample became evident. Here the ratings for the flours were the same under the two methods and took the same ranking as when rated by total average score (Tables IA and IIA).

From the scores on internal characteristics, it would seem that Method 1 is perhaps a more severe color test, since all the flours tested rated a better score for color under Method 2 than under Method 1. The figures for texture and grain again showed greater differences from flour sample to flour sample than between methods, although when these were gathered into rating form the difference between the methods was reflected in the rating order; Method 1, in texture, gave fourth place to flour sample No. 1, and fifth place to flour sample No. 4, while Method 2 reversed these standings. The rankings of the other flours were the same under both methods.

The grain rating under Method 1 gave first place to flour No. 2 while flour No. 5, which ranked first on every other point, took second place. The ratings of the flours No. 1 and No. 4 were reversed by method, Method 1 giving flour No. 1 fourth place and flour No. 4 fifth place; while Method 2 gave flour No. 1 fifth place and flour No. 4 fourth place. The color ratings for flour ranked the same under both methods although the actual score figures for color were higher under Method 2.

Both the scoring figures and the rating figures, viewed compositely and individually, indicate that there is little choice between the two methods for the purpose of testing soft-wheat flours by baking, except for the value of the color test, which is reflected more sharply by Method 1.

The committee gratefully acknowledges the valuable assistance of Laura K. Track in compiling the results of these tests.

REPORT OF THE SUB-COMMITTEE ON METHODS OF TESTING CAKE FLOUR, 1937–38

J. W. MONTZHEIMER, *Chairman*

Centennial Flouring Mills Co., Spokane, Washington

(Read at the Annual Meeting, May 1938)

This year's project was devised especially to compare the results obtained in the laboratories of different collaborators using the A. A. C. C. formula but varying the mixing methods.

Three flours were furnished to this year's committee, all of them commercial cake flours milled in various sections of the country. Each was made from different types of wheat and was considered commercially satisfactory. The approximate protein contents of these flours were 9, 8, and 7%, respectively. All of the ingredients used in this year's work, with the exception of the frozen egg albumen, were furnished to the committee by the chairman. Special care was taken to have all ingredients uniform and of the highest quality. Sugar of an extra fine granulation was chosen, this being sold to the bakers under the grade of Baker's Special. The following five mixing procedures and formulas were used, but the proportions of ingredients were all the same, using the tentative A. A. C. C. formula, Cereal Laboratory Methods, third edition, pages 78–79:

Formula 1: Tentative A. A. C. C. formula using frozen egg albumen.

Formula 2: Tentative A. A. C. C. formula using domestic dehydrated egg albumen.

Formula 3: Tentative A. A. C. C. formula. Cream the sugar and shortening two minutes at second speed. Add frozen-egg albumen slowly to sugar-shortening mix and continue creaming for three minutes. Sift flour, salt, soda, and add to batter alternately with milk, mixing at slow speed for four minutes. Add cream of tartar and mix two minutes.

Formula 4: Tentative A. A. C. C. formula using all dried ingredients. Sift sugar, flour, milk, egg albumen, soda, and salt together three times. Place in mixer. Add shortening; add 100 c.c. water and mix at second speed for two minutes. Add 100 c.c. water and mix three minutes. Add 80 c.c. water and mix four minutes. Then add cream of tartar and mix two minutes.

Formula 5: Tentative A. A. C. C. formula. Place flour, sugar, shortening, soda, and salt together with 150 c.c. milk in mixer and

mix at second speed four minutes. Add 130 c.c. milk and 80 c.c. frozen egg albumen gradually and mix at slow speed for five minutes. Add cream of tartar and mix two minutes.

The three samples of flour were tested by the committee for moisture, protein, ash, pH, viscosity, and flour granulation. Cakes were graded according to present A. A. C. C. official score card. Cake batters were tested for specific volume according to the method outlined by Dunn (Cereal Chemistry **14**: 783). Cake volume was measured in cubic centimeters. Weight of cakes was measured in grams, and specific volumes were computed. Each collaborator reported altitude of laboratory and barometric pressure at time of baking. Type of equipment used in each of the various laboratories was noted.

TABLE I

AVERAGE OF ANALYTICAL RESULTS OF DIFFERENT COLLABORATORS

	Flours		
	A	B	C
Moisture, %	11.5	11.9	11.7
Protein, basis 15% moisture	9.07	7.22	8.21
Ash, basis 15% moisture	.407	.318	.384
pH	5.36	5.00	5.26

Discussion of Results

Variation of moisture reports was caused by drying out of samples during transit and before testing in the laboratories.

Checks between collaborators on well established methods such as ash, protein, and pH were reasonably close.

Only three collaborators reported on viscosity. Results were not concordant.

Granulation tests have not been sufficiently standardized as to method and equipment to obtain valuable results in collaborative work.

Differences in cake character were caused, in this year's work, by difference in pan sizes. Specifications in the Book of Methods do not state whether the figures given for length and breadth of pan apply to top or bottom measurements. Because of the flare in most pans there was a variation in pan size; some collaborators applied their specifications to the top and others to the bottom of the pan.

Cakes were scored differently by different operators, some scoring very severely while others were more lenient.

Comprehensive analysis of reports and summary of results made by Mrs. Laura Track showed that the averages of all of the collaborators for all five formulas gave the flour the same order of rating.

Comments of Collaborators

All of the committee members preferred frozen egg albumen to the powdered egg albumen furnished for this year's work. Six of the committee members preferred the mixing method followed in Formula 5 because it produces a batter of smooth consistency. All collaborators voted to recommend that next year's committee compare commercial-type formulas with our present method of testing.

A majority of the committee were favorable to the specific volume test for testing cake batter because it was valuable in predicting the volume of the finished cake. Several of the committee felt that the test was not as valuable for baking-powder-leavened cakes as it would be in pound or sponge cakes which depend on creaming volume or incorporated air for lightness.

Recommendations for Next Year's Committee

1. That no changes be made on the present A. A. C. C. formula until further studies are completed.
2. That the following dimensions be adopted for the loaf pan: top, $8'' \times 4''$; bottom, $7\frac{1}{2}'' \times 3\frac{1}{4}''$; depth, $2\frac{1}{2}''$.
3. That the following dimensions be adopted as official for round layer cake pan: $8'' \times 1\frac{1}{2}''$.
4. That mixing methods be controlled by stating the number of revolutions of the paddle, rather than the mixing time. (There is a variation in speed of the mixers used by different committee members.)

Recommendations for Future Study of the Cake Committee

1. That type photographs of both loaf and layer cakes be made and circulated among committee members for criticism, in order to set up definite photographic standards in grading the interior and exterior properties of cakes.
2. Since this year's project shows many differences in baking results, which may be caused by variations of baking conditions, the committee recommends that a close record be kept of: (1) baking time, (2) of temperature in the oven 2 inches directly over the cake pan—this can be accomplished by suspending a thermometer in the oven two inches above the baking cake, (3) baking losses, with the idea of bringing losses among collaborators to the same magnitude. (This year's committee showed baking losses from 25 to 70 g. for cakes baked in different laboratories with the same ingredients and formula and supposedly the same baking conditions.)
3. Standard cake liners should be used by the committee.
4. Future committees should report pH on finished cake.

5. Since there is a demand by certain chemists for investigation of commercial-type formulas, it is recommended that a study of commercial formulas, which have been assembled by this year's committee, be carefully made during the first part of the year. One or more of these formulas should be set up in the laboratories of the various collaborators, so that a comparison may be made on routine work between the new formula being investigated, the present A. A. C. C. formula, and the formula habitually used for testing purposes in the respective laboratories. It is suggested that the comparison be made under laboratory conditions, at least once each week for the first three months, and after the formula has been studied in this manner it be corrected and standardized for consideration of the committee as a whole. Any formulas receiving especially favorable rating could then be used for collaborative work during the remainder of the year. In this manner the value of commercial types of cake formulas for testing purposes could be compared with that of our present method and the committee would then be in a position to recommend either the continuance of the A. A. C. C. method or a good type of cake formula as a substitute for it.

Membership

J. W. Montzheimer, Chairman	J. R. Davies	O. E. Stamberg
W. E. Stokes	E. P. Walker	F. J. Coughlin
R. W. Mitchell	W. C. Rohrbaugh	F. D. Machon
	H. R. Fisher	L. D. Whiting

The committee wishes to acknowledge the assistance of Donald Wade of Mr. Coughlin's staff and of Mrs. Laura Track of Dr. Stokes's staff.

SUPPLEMENT TO REPORT OF 1937–38 COMMITTEE ON TESTING CAKE FLOUR

W. E. Stokes and Laura K. Track [1]

Standard Brands, Inc.

Royal Baking Powder Factory, Brooklyn, N. Y.

Ratings for baking qualities of the several flours were based upon the score figures allocated by the individual collaborators. Separate ratings were given for external score, internal score, total score, and volume. Ratings were arrived at by giving "first place" to highest score, "second place" to second-highest score, and "third place" for low score. The ratings thus obtained for volume, external and internal and total scores, were ascertained by totaling the number of ratings in each place for each flour, thus setting up a final place rating. For example under Formula 1, one of the collaborators scored the flours for external baking characteristics as follows:

[1] Report of 1937–38 sub-committee on Testing Soft-Wheat Flours.

		Rating
Flour A	25	1
Flour B	21	2
Flour C	21	2

For internal, as follows:

		Rating
Flour A	52	2
Flour B	56	1
Flour C	51	3

For total score, as follows:

		Rating
Flour A	77	1
Flour B	77	1
Flour C	72	2

For volume, as follows:

		Rating
Flour A	768	1
Flour B	702	3
Flour C	727	2

Thus the total of "first" ratings for flour A was 3, and the number of "second" ratings, 1. Flour B totaled two "first" ratings, one "second," and one "third." Flour C showed no "first" rating, three "second," and one "third" place.

Flour A, having scored the greatest number of "first" ratings and no "third" rating, was given a final place rating of one. Flour B with two "first" ratings, one "second," and one "third," scored a final-place rating of two. Flour C showed out in a final "third" place rating with no "first," three "seconds" and one "third" place rating.

The final composite place ratings were obtained by totaling, as in the foregoing, the "final place ratings" by individual collaborators' scores. Of the ten collaborators, three gave first ratings to flour A, six first place to flour B, and one gave first rating to flour C.

Five of the collaborators by their scores rated flour A for second place, four gave flour B second place, and two gave second place to flour C. Final consideration of these place ratings brought the final place ratings:

First place	Flour B
Second place	Flour A
Third place	Flour C

This plan was carried through for the three flours, five formulas, ten collaborators, arriving at the final composite rating under all formulas as:

Flour A	2
Flour B	1
Flour C	3

Place ratings given the flour samples on an average basis rated in the same order under formulas 3, 4 and 5, as when computed on the composite basis, but under formulas 1 and 2, the final *average* place ratings were:

Flour A	1
Flour B	2
Flour C	3

TEN COLLABORATORS' AVERAGE SCORE FOR THREE FLOUR SAMPLES BY FIVE MIXING METHODS

A. A. C. C. Cake Baking Tests for Testing Soft-Wheat Flours

Perfect score	Formula 1, Flours			Formula 2, Flours			Formula 3, Flours			Formula 4, Flours			Formula 5, Flours		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
A. External															
10—1. Symmetry	8.8	8.4	8.2	8.5	7.8	7.7	8.5	8.7	8.3	8.3	8.2	7.6	8.8	8.4	8.3
15—2. Volume	13.2	11.4	11.0	12.0	11.4	11.1	12.9	13.4	12.5	12.2	12.5	11.3	13.8	13.0	12.1
5—3. Crust sugariness	3.7	3.8	3.5	3.5	3.6	3.5	4.0	4.0	3.8	3.9	3.9	3.6	3.7	3.9	3.4
Total external	25.7	23.6	22.7	24.0	22.8	22.3	25.4	26.1	24.6	24.4	24.6	22.5	26.3	25.3	23.8
B. Internal															
15—1. Texture tenderness	11.0	11.7	12.0	10.5	10.9	9.8	11.7	12.5	11.3	11.4	11.6	10.0	12.1	12.5	11.9
15— Silkiness	11.5	12.2	10.8	11.0	11.2	9.4	11.3	12.9	11.1	11.5	11.5	10.1	11.9	12.8	11.7
25—2. Grain	17.6	18.6	16.8	18.0	17.9	15.4	18.0	20.0	18.7	18.9	18.7	17.7	19.7	21.2	20.4
15—3. Color	11.1	13.1	11.5	10.0	11.0	9.6	11.8	13.6	12.1	10.1	12.0	10.1	11.7	13.6	12.2
Total internal	51.2	55.6	51.1	49.5	51.0	44.2	52.8	59.0	53.2	51.9	53.8	47.9	55.4	60.1	56.2
100 total score	77.0	79.0	74.0	74.0	74.0	67.0	78.0	85.0	78.0	76.0	78.0	70.0	82.0	85.0	80.0
Sp. volume of batter	1.13	1.08	1.07	1.13	1.09	1.08	1.13	1.15	1.13	1.13	1.15	1.10	1.15	1.15	1.11
Weight of cake	286	284	286	286	285	285	287	286	288	287	286	285	289	285	286
Volume of cake, *c.c.*	811	770	755	798	762	758	799	800	784	798	804	767	826	805	793
Sp. volume of cake	2.83	2.71	2.65	2.76	2.68	2.65	2.81	2.81	2.74	2.77	2.79	2.71	2.90	2.81	2.78
Rating															
By external score	1	2	3	1	2	2	2	1	3	1	1	2	1	2	3
By internal score	2	1	2	2	1	3	2	1	2	2	1	3	3	1	2
By total score	2	1	3	1	1	2	2	1	2	2	1	3	2	1	3
By volume	1	2	3	1	2	3	2	1	3	2	1	3	1	2	3
Number of place ratings=															
First	2	2	0	3	2	0	0	4	0	1	4	0	2	2	0
Second	2	2	1	1	2	2	4	0	2	3	0	1	1	2	1
Third	0	0	3	0	0	2	0	0	2	0	0	3	1	0	3
Final place rating	1	2	3	1	2	3	2	1	3	2	1	3	2	1	3

FINAL PLACE RATINGS

ACCORDED THREE FLOUR SAMPLES UNDER FIVE FORMULAS BY COLLABORATORS

Collaborators	Formula 1			Formula 2			Formula 3			Formula 4			Formula 5		
	Flours			Flours			Flours			Flours			Flours		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
E. P. Walker	1	2	3	2	1	3	2	1	3	2	1	3	2	1	3
W. C. Rohrbaugh	2	1	3	–	1	2	3	1	2	2	1	3	3	1	2
J. W. Montzheimer	2	1	3	3	1	2	1	2	3	1	3	2	1	2	3
R. W. Mitchell	3	1	2	2	1	3	3	1	2	2	1	2	2	1	3
Donald Wade	1	2	2	1	2	2	2	1	3	1	1	2	2	1	3
Olaf Stamberg	1	2	3	2	1	3	2	1	3	2	1	3	2	1	3
W. E. Stokes	2	1	3	2	1	3	2	1	3	3	1	2	2	3	1
F. D. Machon	2	1	3	2	1	3	3	1	2	3	2	1	3	1	2
H. R. Fisher	3	2	1	1	2	2	2	1	3	2	1	3	2	1	3
L. D. Whiting	2	1	3	1	3	2	3	1	2	1	2	3	1	1	2
Number of place ratings:															
First	3	6	1	3	7	0	1	9	0	3	7	1	2	8	1
Second	5	4	2	5	2	5	5	1	4	5	2	4	6	1	3
Third	2	0	7	1	1	5	4	0	6	2	1	5	2	1	6
Final composite place rating	2	1	3	2	1	3	2	1	3	2	1	3	2	1	3

REPORT OF 1937–38 SUB-COMMITTEE ON METHODS OF TESTING SELF-RISING FLOURS

O. E. GOOKINS

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(Read at the Annual Meeting, May 1938)

The personnel of the 1937–38 sub-committee on Methods of Testing Self-Rising Flours was as follows: R. A. Barackman, L. E. Leatherock, Harold McGhee, Elizabeth McKim, H. V. Moss, Grant W. Pearcy, C. C. Walker, and O. E. Gookins, chairman. Five members of this committee are connected with milling concerns and three with chemical companies supplying self-rising ingredients.

A general summary of work done by previous committees on testing self-rising flours was prepared and presented to the members of the committee. A questionnaire was also sent out covering various items suggested by former committees, and tentative subjects for study this year. The replies brought out the following points:

1. Everyone approved continuing with the 1935 biscuit recipe (flour 15% moisture basis, 227.7 g.; soda 3.4 g.; mono-calcium phosphate 4.3 g.; salt 4.6 g.; hydrogenated shortening 30 g.); however, two members suggested less shortening for soft flours.

2. Five approved the method of mixing (as given by the 1933–34 committee, Cereal Chemistry **12**: 162). Suggested improvements were:

Make the method faster and more usable in practice.
Obtain better fat distribution.
Omit one fold and one roll of dough.
Study further the variables.

3. Definite specifications for a possible flour standard were given in only one instance, the general feeling being that we do not have enough experience to make recommendations. Ash, protein, pH, color, absorption, viscosity, moisture, and granulation were all suggested as factors to be considered. One suggestion was to have at least three hard and three soft wheat flour standards.

4. No one had a satisfactory method for flour color determination that could be duplicated in other laboratories.

The program of work taken up by this year's committee was in the nature of a study of various items affecting baking conditions and the scoring system. The work was done as individual assignments, with committee collaboration on some of the problems.

R. A. Barackman reported on the effect of one fold, two rolls of dough vs. two folds, three rolls. Using a soft wheat patent flour at 64% absorption, and with A. A. C. C. formula and procedure, the following data (average of three trials) were obtained:

	Wt. dough	Oven loss	Biscuit vol.	Specific vol. *c.c. biscuits/ g. dough*
	g.	%	*c.c.*	
1 fold, 2 rolls	156	16.0	348	2.23
2 folds, 3 rolls	173	16.2	373	2.16

It will be noted that the one-fold, two-roll method allows 10% lighter doughs to be cut, 6.7% less volume of biscuits, and 3% lighter biscuits in terms of specific volume. The grain of the biscuits made by the one-fold, two-roll method was somewhat easier to judge, but substantially the same score would be assigned to biscuits from both methods.

Barackman also reported on the variations in biscuit bakings due to ovens. His data for electric vs. gas-oven baking (average of five replicate bakes in each) follow:

Oven	Bake temp.	Wt. of dough	Oven loss	Biscuit vol.	Spec. vol.	Crust score
		g.		*c.c.*	*c.c./g.*	
Electric	475° F.	161	14.7%	353	2.194	9
Gas	475° F.	160	16.7%	352	2.202	10

Both ovens had automatic temperature controls, which were checked by thermometer readings. Gas or electric oven baking causes no appreciable difference in volume of baked product. The biscuits baked in an electric oven had a somewhat paler crust color compared to those in the gas oven. A greater oven loss was obtained with gas. No other differences in points of scoring biscuits were observed. These findings agree with the 1934–35 committee report in regard to oven losses, but not in the matter of volume. Their report stated that "Higher oven losses were accompanied by greater specific volumes." This discrepancy is evidently due to the fact that the former conclusion was based on the data of several collaborators who baked in different laboratories with one type of oven only. The present report has to do with the careful comparisons by one operator within a single laboratory.

C. C. Walker reported on a study of water absorption as related to biscuit baking. Using a short patent, soft-wheat flour, which showed a farinograph absorption of 56.3% at 15% moisture, and baking by A. A. C. C. method, the following specific volumes were obtained for varied amounts of water:

Absorption, % (basis 15% moisture)	No. replicate bakes	Average spec. vol.	Comments
56.3	1	1.97	Dry
57.0	1	1.97	Dry
60.0	6	2.02	Good
61.0	2	1.87	Good
61.5	2	1.88	Good
62.0	3	1.87	Slack
63.0	5	1.90	Slack
65.0	1	1.81	Very slack

The specific volumes did not vary much with different absorptions. The color and texture were about the same, except in the very dry doughs where the inside of the biscuit was too compact. Optimum absorptions, considerably higher than the farinograph absorption, confirmed results obtained by the 1934–35 committee on Methods of Testing Self-Rising Flours.

Elizabeth McKim and H. V. Moss had as their problem the study of definition and evaluation of various items on the score card. A paper is to be presented by them on this subject.

Grant W. Pearcy continued his work of last year on preparation of permanent color standards for biscuits. His report on color determinations will also be given separately.

One suggestion made to the committee was to use a sheet of metal lath (plaster lath) in the oven under the baking pan. This seemed to improve the evenness of the bake.

This concludes the report of this year's work, which may be summed up as follows:

1. One-fold, two-roll procedure is preferable to two-fold, three-roll method.
2. There is little difference between gas and electric ovens as affecting biscuit score, except the crust color.
3. Absorption variation of small percentages is not particularly critical in biscuit scoring.
4. Tentative definitions of score-card terms are outlined.
5. Series of photographs, representing a range of textures, are proposed for reference standards.
6. A method of preparing semi-permanent color standards for biscuit scoring is given along with collaborative tests.

Items 4 and 5 are covered in papers by McKim and Moss, and Item 6 by Pearcy.

Next year's committee may find it advisable to do more work on the actual technique of baking, and to try out, by collaborative baking, the definitions and texture and color scoring methods presented in this year's committee work. It is also suggested that work be done on a supplementary formula for indicating the best color possible in lower grades of flour.

The percentages of phosphate and soda now in use are those recommended by the 1931–32 committee. The ratio of soda to monocalcium phosphate is 0.791. This is satisfactory for patents, but for lower grades of flour the biscuit color is unduly dark and creamy. A ratio of soda to phosphate in the range of 0.73 to 0.75 produces much different results on flours of above types, and is, perhaps, more in line with commercial practice.

Another supplementary test which might merit some investigation is flour granulation as a means of further classifying flours for self-rising purposes.

Previous Reports Cited

Walter, H. G.
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STUDY OF DEFINITION AND EVALUATION OF VARIOUS ITEMS ON SCORE CARD[1]

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(Read at the Annual Meeting, May 1938)

As a part of the activities of the sub-committee on Methods of Testing Self-Rising Flours the assignment of defining and evaluating the five score items on the A. A. C. C. biscuit score card was allocated to two of the collaborators. In an endeavor to clarify the meanings of the various score items, the collaborators enlisted the aid of the other committee members and circulated a questionnaire requesting individual definitions and methods now employed by the committee members for evaluation of scores. From the replies, the following consensus on definitions and methods has been reached:

Grain

Definition: An index of the physical structure of the biscuit crumb with reference to (*a*) the *size*, *shape* and *homogeneity* of *cells*, and (*b*) the *thickness* of the *cell wall*.

Method of evaluating: Judging by visual observation of horizontal or vertical sections of cold biscuits with or without reference to a control.

Tenderness

Definition: A measure of the resistance to shear and compression of the biscuit crust and crumb.

Method of evaluating: Personal judgment of the feel of a biscuit when broken, compressed, or eaten with or without reference to control.

Flavor

Definition: The quality of a flour which affects its smell and the taste and aroma of a biscuit baked from it.

Evaluation: Organoleptic comparisons with reference to a control involving: (*a*) the smell of a flour at room temperature or when mixed in hot water, and (*b*) the smell and taste of hot or cold biscuits. Scoring to be made with consideration of the following points given by R. A. Barackman in the 1933–34 self-rising flour committee report

[1] Report of 1937–38 sub-committee on Testing Self-Rising Flours.

(Cereal Chemistry **12**: 164); the extent of mark-down to be dependent upon the judgment of the technician with reference to a sound, fresh, properly leavened standard flour and the numerical score to be accompanied with suitable descriptive terms:

	Desirable	*Undesirable*
Flour condition	Sweet	Rancid, musty
Flour quality	Sweet	Wheaty, starchy
Leavening	Neutral	Acid or sour, alkaline or soapy
Salt	Pleasing	Salty, flat
Eating quality	Good or chewy	Doughy, dry and crumbly

Crumb Color

Definition: A comparative expression of the color of the cut surface of the biscuit crumb.

Evaluation: Visual comparison with reference to one or more controls.

Volume

Definition: The average specific volume of the baked biscuit on a basis of the weight of dough with reference to a standard.

Evaluation: Determination of the volume of biscuits by seed displacement divided by the weight of dough; this calculated to a ratio of 2.00 as standard and weighted to 40 on the score card, *viz:*

$$\frac{\text{c.c. biscuit}}{\text{g. dough}} / 2.00 \times 40 = \text{volume.}$$

Discussion

Grain: There is good agreement on definition, with uniform stress on characteristics of the cells and cell walls. Judging grain depended in many cases wholly on the conception of the technician without reference to a control. The need of standardization in judging grain is shown by comments of the collaborators suggesting preparation of reference standards embodying:

(*a*) Photographic plates
(*b*) Ink prints
(*c*) Preserved biscuit sections or physical measurements including:
(*d*) Moisture absorption
(*e*) Sand penetration

All of these have been suggested or used with more or less success in bread and cake work.

Tenderness: Agreement on definition is good. Method of evaluation depends in every case on the response of a biscuit to breakage or

compression of the crumb by hand, or to the feel of the biscuit when eaten, both the crust and crumb being considered. In no case is "tenderness" being gauged mechanically. Some little experience in this collaborator's laboratory on a means of judging tenderness mechanically indicated that a reliable measure depended upon a large number of determinations of carefully prepared biscuit sections, and made the particular method tried impractical as a routine procedure. In spite of the evident uncertainty of the method now generally practiced, it appears to be the only practical one available; reference to a control is deemed desirable as some basis of comparison.

Flavor: The A. A. C. C. score card divides the flavor score into "flour quality" and "eating quality" as distinct from tenderness. Attention is called to the characteristics of "eating quality" in the tabulation of points to consider in judging flavor. Clarification of this point in the minds of the committee members is needed and consideration should be given to whether "eating quality" should not be included as a tenderness characteristic rather than a property of flavor.

Judging the smell of the flour prior to baking is deemed necessary since foreign odors or contamination are frequently encountered during mixing and the consumer is affected by this observation.

Color: The need for standardization of color score has been recognized by previous committees; this is brought out again by replies to the questionnaire which suggest as controls:

(*a*) Biscuits baked concurrently with the unknown from one or more flours varying within known limits in color characteristics
(*b*) Reference to color disks, or
(*c*) Use of preserved biscuit sections

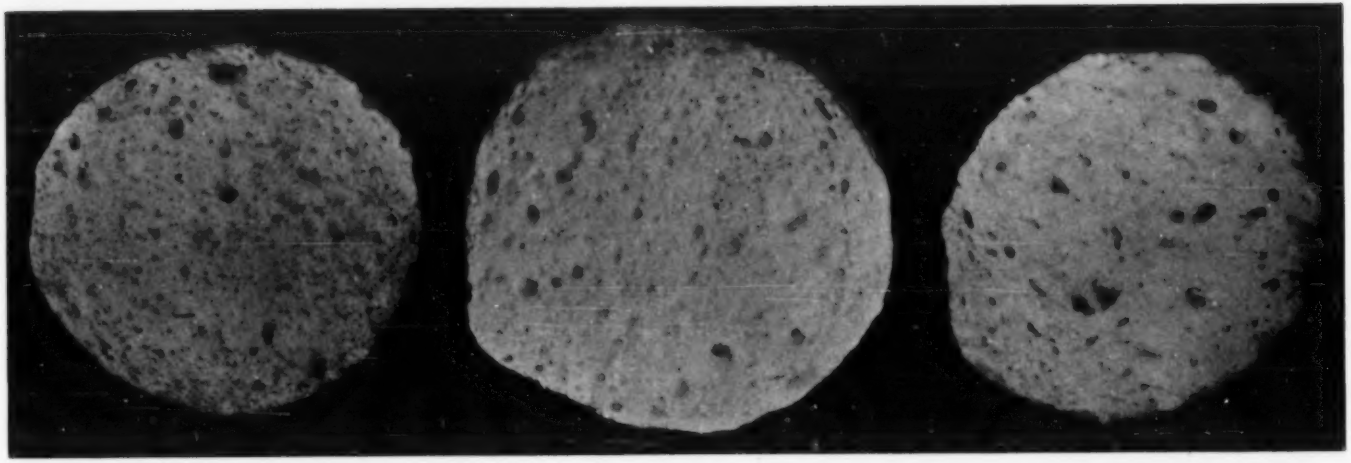
This last method was investigated in a preliminary way by the 1936–37 committee and because of favorable indications work on the preparation of suitable preserved standards has been continued this year by Grant Pearcy.

A value of 20 is attributed to crumb color in the A. A. C. C. biscuit score card and several of the committee members have pointed out that from a commercial viewpoint, this characteristic is of greater relative importance. From the experience of these collaborators, this appears to be a justified criticism, and it is suggested that consideration be given to revision of the A. A. C. C. biscuit score, with a view to increasing the importance of the crumb color score.

Volume: This is the only item in the biscuit score card which is reasonably well standardized. In a series of tests made by the 1933–34 committee on self-rising flours it was shown that three collaborators agreed within 2.3% on individual specific-volume determinations after

PLATE I

Typical biscuit textures from flour types



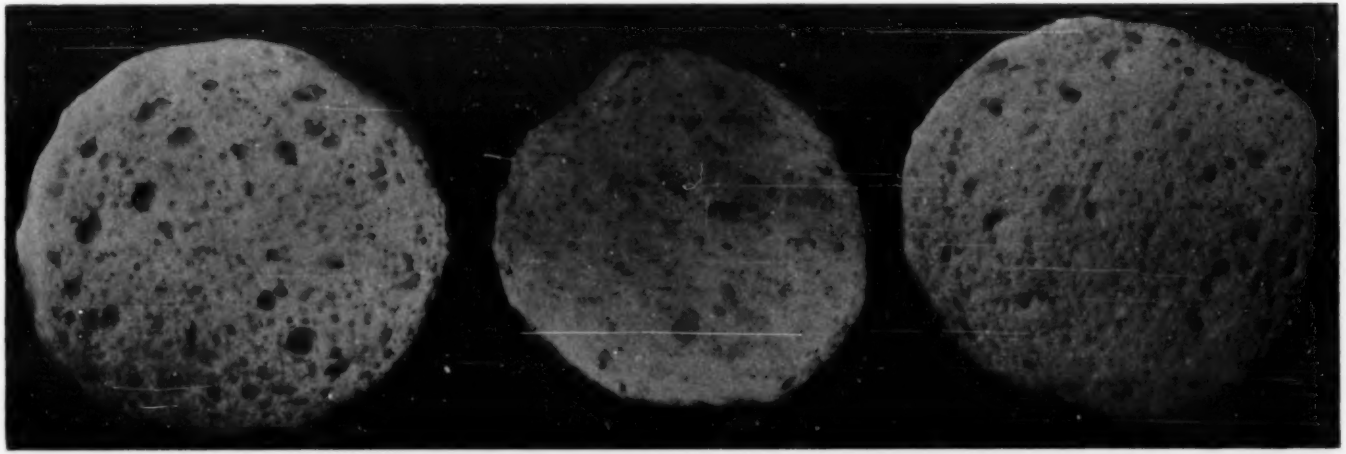
"Coarse"
Soft Wheat
2nd Clear

"Standard"
Soft Wheat
40% Patent

"Close; Bready"
Hard Wheat
70% Patent

PLATE II

Typical biscuit textures from leavening variations



Open
Soft Wheat
Straight
Excess Leavening

Soft Wheat
40% Patent
Excess Leavening

Standard

Standard
Leavening

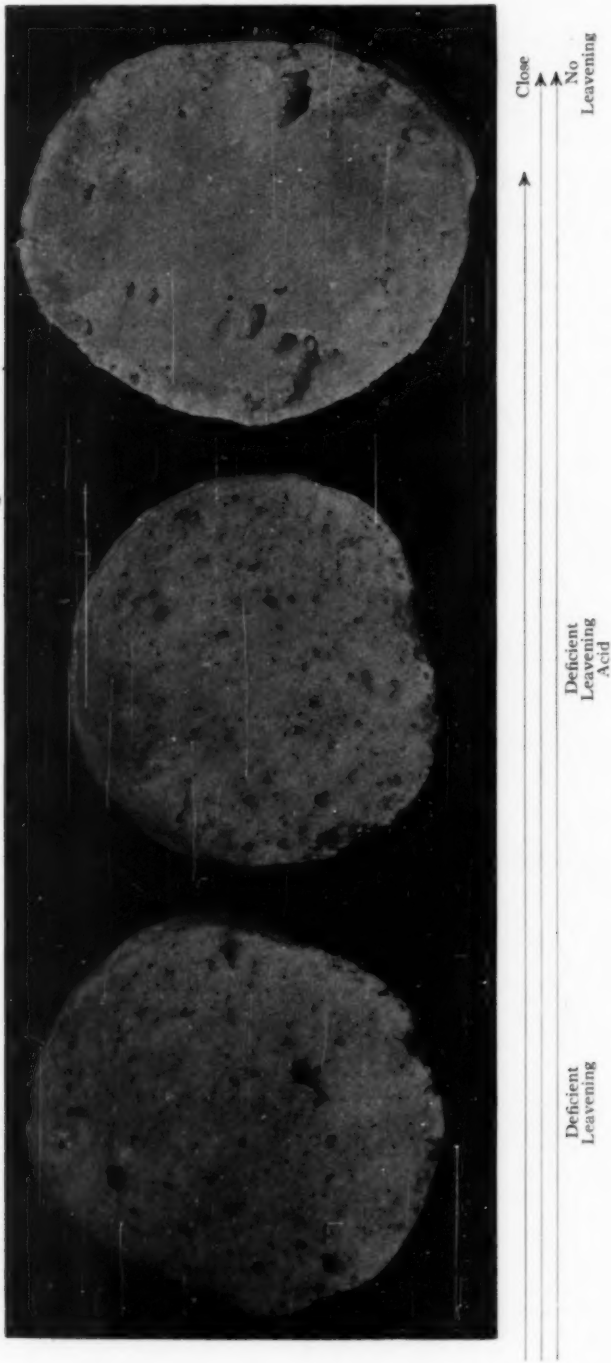


PLATE II—*Continued*

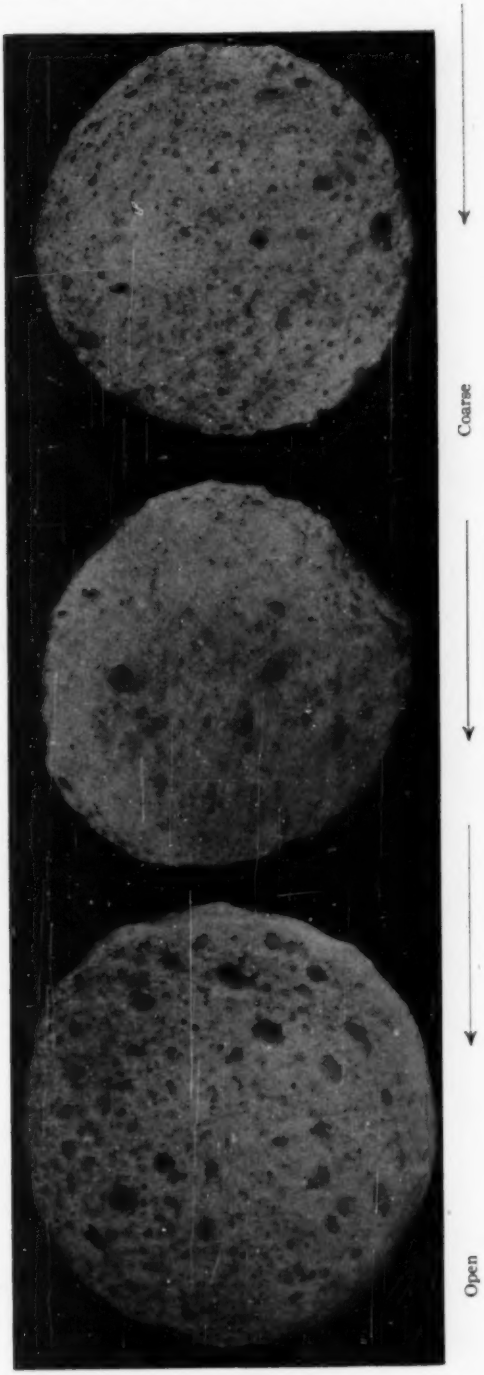


PLATE III

Proposed standard texture types

PLATE III—*Continued*

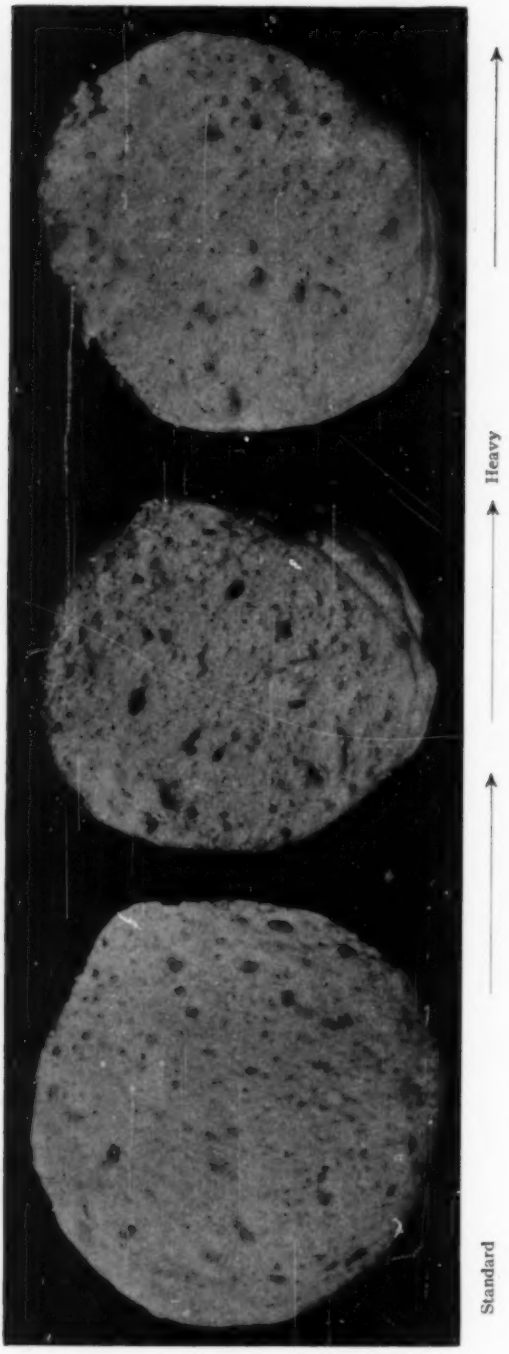


PLATE III—*Continued*

preliminary calibration of measuring apparatus by means of wooden biscuit dummies of known volume.

The value of 40 now allotted to "volume" on the A. A. C. C. biscuit score appears to be given a disproportionate credit. Whereas volume is recognized to be a good relative index of lightness and desirable texture, it is also recognized that in many of the real biscuit-eating territories, a large, high biscuit is not necessarily a criterion of quality and furthermore it has been the experience of these collaborators that "volume" is subject to less variation with changes in self-rising flour quality than some of the other factors in the score. It is therefore suggested that consideration be given to revising the "volume" score downward, giving preference to color, grain, and tenderness in the order named.

In carrying out the assignment given us by the chairman of the Sub-Committee on Methods of Testing Self-Rising Flours, we have endeavored to prepare a set of photographic plates designed to illustrate typical biscuit textures with a view to establishing ready reference standards for judging biscuit grain or texture. The plates are self-explanatory and represent a suggestion of what may be accomplished. It will be observed that the pictures made so far are of horizontal biscuit sections; it is acknowledged that vertical sections are equally desirable and continuation of the work contemplates making pictures of these as well.

Plates I and II represent textures encountered with flour types and leavening variations respectively. Plate III is a composite of Plates I and II arranged to show trends in grain change. An increasing heaviness and closeness of grain is illustrated by the biscuits to the right, while the biscuits to the left of the standard illustrate increasing "coarseness" and irregularity of "open" texture.

It is a question whether normal judges of biscuit grain are able to recognize the differences in the pictures, with which we have become thoroughly familiar after numerous attempts at portraying photographically what we were able to observe in the "live" biscuit. As the next step in the development of standard grain plates it is proposed to supply the committee members with shuffled sets of pictures, asking that they arrange them in the order of their conception of quality and that they assign a numerical score to each biscuit. It is hoped that by this procedure a set of typical standard grain or texture plates may be established in which the range of the grain score will be shown.

CONVENIENT CRUMB COLOR STANDARDS FOR SELF-RISING FLOURS [1]

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H. W. PUTNAM

Igleheart Brothers, Incorporated, Evansville, Indiana

(Read at the Annual Meeting, May 1938)

An unpublished progress report on the preparation and collaborative testing of convenient crumb-color standards for self-rising flours was read at the annual meeting, 1937, as a sub-project of the committee on Cake Baking Tests and Self-Rising Flours. That report indicated that reasonable agreement seemed possible between collaborators who were measuring biscuit crumb color in terms of a somewhat absolute scale instead of in descriptive terms or by means of indefinite numerical ratings.

Work this year was planned largely to deal with several matters proposed at that meeting, and included: (1) improvement of the standard board, (2) the further collection of collaborative data, (3) determination of changes during the year in the standards originally prepared, and (4) determination of the effect of sunlight on the collodion preserved biscuit slices. Work done previously was reviewed.[2] Bailey's method for scoring rye bread, privately communicated, was adapted to the preparation of standards for biscuit crumb color.

Preparation of the " Color Charts "

The "color charts" consist essentially of black cards, with two closely parallel rows of windows, fastened to a piece of colorless clear thin glass. To the back of one row are fastened thin dried slices of biscuit crumb preserved in dilute collodion. The unknown is viewed through the openings in the adjacent row and is identified by the number of the standard matched.

Seven flour blends, selected to give an evenly spaced range of crumb colors, were baked, with every precaution taken to prevent streaks.

[1] Sub-committee report, 1937–38 Committee on Testing Self-Rising Flours.

[2] L. H. Bailey, Report of the committee on testing rye flour, Cereal Chem. 13, 770–772, 1936. J. C. Baker, H. K. Parker, and F. B. Freese, The measurement of color in flour and bread by means of Maxwell discs, II, Cereal Chem. 12, 17–24, 1935. Emily Grewe, W. K. Marshall, and C. G. Harrel, A method of measuring color in bread, Cereal Chem. 6, 60–68, 1929. M. C. Markley, Method of preserving bread for permanent grain judging standards, Cereal Chem. 11, 200, 1934. L. D. Whiting, Report of the 1935–36 committee on cake-baking tests and self-rising flours, Cereal Chem. 13, 736–745, 1936.

Slices cut from the cold biscuits were trimmed to the desired size and thoroughly air-dried between clean, white blotters, under enough weight to keep them flat without crushing. These dried "chips" were preserved by dipping for eight seconds in a solution of 30 c.c. U. S. P. flexible collodion in 75 c.c. ethyl alcohol and 195 c.c. ethyl ether and redrying between the blotters under the weight. The slices were attached to the glass by means of liquid adhesive and the backs covered with strips of adhesive tape which were coated with several applications of clear shellac. This gives a standard color chart capable of being handled without destruction or soiling.

Experimental

Four carefully matched color charts were used in the collaborative work in 1937. A color chart and four samples of leavened flour containing shortening were sent to each laboratory. Directions were given for hand mixing the biscuits and for preparing the slices for color scoring.

The flours were baked and scored independently by two or more persons in each of the collaborating laboratories. The charts were then returned to the authors' laboratory where samples of the same flours were baked and scored against each color chart respectively and independently by four individuals. One color chart was returned too late to be included in the averages of all observations (Table I).

TABLE I

AVERAGES OF CRUMB COLOR SCORING RESULTS ON 1937 SAMPLES AS REPORTED BY ALL COLLABORATORS

Chart No.	Flour A	Flour B	Flour C	Flour D
2	6.2	2.9	1.5	4.7
3	6.0	2.9	1.5	4.8
4	5.9	2.6	1.3	4.8

Since results in Table I may have been influenced too much by the skill developed in the authors' laboratory, the following averages are given to show the results obtained in the collaborating laboratories alone.

TABLE II

AVERAGES OF CRUMB COLOR SCORING RESULTS ON 1937 SAMPLES AS REPORTED BY ALL COLLABORATORS EXCEPT THOSE IN AUTHORS' LABORATORY

Chart No.	Flour A	Flour B	Flour C	Flour D
2, in Lab. 2	6.7	3.0	1.7	4.7
3, in Lab. 3	6.1	3.2	1.9	5.2
4, in Lab. 4	6.0	2.8	1.4	5.2

The results in Table II reflect both the differences in judgment of the collaborators and any slight differences between the charts.

These four samples of flour previously used in 1937 were preserved in tightly closed cans and baked after a period of twelve months. They were scored against the original color charts, which had been kept in the dark. Results (Table III) showed a general dropping of color values from 1½ steps in the darker end of the board to 3 steps in the lighter end.

TABLE III

AVERAGES OF RESULTS OBTAINED AFTER TWELVE MONTHS' STORAGE, USING FLOURS AND CHARTS PREPARED IN 1937
(Compare with Table I)

Chart No.	Flour A	Flour B	Flour C	Flour D
2	7.5	5.0	4.5	6.0
3	7.2	5.5	5.0	6.0
4	7.5	5.5	4.3	6.5

In 1938 a single new color-standard board was used by all the collaborators. Special precaution was taken to keep this in a light-proof box when not in use. Two samples of flour, containing baking chemicals and shortening, were baked by collaborators and scored against this standard board. The results (Table IV) show gratifying agreement.

TABLE IV

RESULTS OF COLLABORATIVE STUDIES, 1938, SAME STANDARD USED BY ALL COLLABORATORS

	Collaborators								
	A	B	C	D	E	F	G	H	I
Flour E	1	1½	1¼	1½	1	1	1½	1	1
Flour F	2	2	2	2¼	2	2	2½	2	1½

Average for Flour E 1.19
Average for Flour F 2.03

In order to determine the effect of sunlight on the preserved slices two identical color charts were prepared and used in scoring two samples of flour. One of the two charts was immediately placed in a light-proof box, and the other exposed continuously to daylight and sunlight on a window sill. At short intervals the two test flours were baked and scored against the two color-standard charts. Within two days the chart exposed to the sunlight showed lighter and at the end of one week had dropped 2½ steps. At the end of one month readings had not changed further.

TABLE V

READINGS USING STANDARD CHARTS WITH ONE KEPT IN DARKNESS AND ANOTHER EXPOSED TO SUNLIGHT

	Stored in darkness			Exposed to sunlight		
	2 days	1 week	1 month	2 days	1 week	1 month
Flour E	1	1	1	2	3½	3½
Flour F	2	2	2	3	5½	5½

Conclusions

This method of scoring crumb color of biscuits is satisfactory for use within a short period of time if special attention is given to protecting the standard board from light. The method, however, is not satisfactory over an extended period of time.

Acknowledgment

The authors wish to acknowledge the help given by the members of the committee and their associates who acted as collaborators.

1937–1938 REPORT SUB-COMMITTEE ON TESTING BISCUIT AND CRACKER FLOURS

PEARL BROWN, *Chairman*

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(Read at the Annual Meeting, May 1938)

The 1937–38 sub-committee planned its work to carry out, in so far as possible, the recommendations of its predecessor (Cereal Chemistry **15**: 35–48) as follows:

1. "That more work of the type herein reported be done to build up a greater volume of data to correlate more accurately the laboratory analyses of biscuit and cracker flours with their shop performance."

2. That "experimental work be undertaken to explain, and if possible correct, the apparent discrepancy between the baking test and the viscosity test for determining the relative strengths of biscuit and cracker flours."

Procedure

The committee followed the procedure outlined by the previous committee. Committee members submitted analyses of flours with which they were familiar and which might be suitable for study. From

these lists four flours were selected. Each flour, so coded that its identity was hidden, was submitted twice to each committee member. The first time, determinations of moisture, ash, protein, and viscosity were made, and from these data the flours were classified as sponge or dough flours. The second time committee members were asked to run baking tests alone and to classify the flours from the results.

Crackers were made in the shop, also, according to the method used by Reiman (1938); that is, the two sponge flours were baked separately with the two dough flours, making four bakes. This series was tested three times at intervals of from three to four weeks. After each bake, a one-pound package of each type of cracker was sent to each committee member for scoring. On the first two bakes topping salt was omitted from the crackers. One member was asked to analyze the crackers for moisture, protein, and fat content, and another was asked to determine the shortometer values.

For the sake of continuity, the four flours used in this study are designated as 5, 6, 7, and 8, respectively. Table I shows the type of wheat from which each flour was milled.

TABLE I

AVERAGE ANALYTICAL RESULTS ON THE FLOURS STUDIED

Sample no.	Used for	Wheat type	Ash at 15% H^2O	Protein at 15% H^2O	Viscosity		
					No acid	1 c.c.	7 c.c.
5	Sponge	Red	0.396	8.05	16.4	29.6	58.5
7	Sponge	Red	0.398	8.45	16.0	38.2	70.8
6	Dough	Red and white	0.378	7.86	16.6	33.3	56.0
8	Dough	Red and white	0.358	7.75	18.2	34.8	46.5

The methods for determining moisture, ash, and protein were the usual ones. The method for the viscosity test was that of Bayfield (1936), procedure 2.

The shortometer test was the same as was used last year, Reiman (1938), with the exception that only 52 readings were made on the first series of four crackers, 168 on the second, and 145 on the third series.

Commercial Cracker Bakes

The procedure of the previous committee was followed as closely as possible. The differences were essentially those that might be expected to be encountered in different shops. Three series of bakes were made at intervals of from three to four weeks. Each series was composed of four five-barrel doughs. Each of the two sponge flours was baked with each of the two dough flours. Sixty-percent sponges were made, 50% of the total flour being incorporated in the sponge,

and 50% in the dough stage. The last cracker made was an exception. In it, 60% or all of the sponge flour was used in the sponge, and 40% or all of the dough flour in the dough.

The sponges, set at 70° F., were fermented 20 hours in a fermentation room maintained at 84° F. with the relative humidity between 50% and 60%. On the first bake, because of a condition in the shop, cracker No. 4 had 22 hours in the sponge stage. In the last bake, cracker No. 5, the sponge was set at 68° F.

Other conditions were maintained as nearly uniform as possible throughout the experiments. The doughs were mixed, fermented,

TABLE II

CLASSIFICATIONS OF FLOURS MADE ON BASIS OF ANALYTICAL TESTS INCLUDING VISCOSITY

Collaborator	Flour 5	Flour 6	Flour 7	Flour 8
ARMUTH	Dough	Dough	Sponge	Dough or cookie
EVERT	Dough or sponge	Dough or sponge	Sponge	Dough or cookie
HOLLINGSHEAD	Dough	Dough	Sponge	Strong sweet goods and cracker dough
KNUDSON	Dough or hard sweets	Dough	Sponge	Cookie-cracker dough
LOVING	Sponge dough or hard sweets	Dough—could be sponge	Sponge	Dough
OPPEN	Dough or sponge	Sponge or dough	Sponge	Dough or sweet goods
SKAER	Dough	Dough	Sponge	Dough
TRIEBOLD	Dough or sponge	Sponge or dough	Sponge	Dough

TABLE III

CLASSIFICATIONS OF FLOURS MADE ON BASIS OF BAKING TESTS

Collaborator	Flour 5	Flour 6	Flour 7	Flour 8
ARMUTH	Dough	Dough	Sponge	Cookie
EVERT	Dough and cookie	Dough and cookie	Dough or cookie	Cracker sponge
HOLLINGSHEAD	Strong sweet goods and cracker dough	Very good cracker dough	Weak sponge or strong dough	Cookie
KNUDSON	Dough and hard sweet	Dough	Sponge	Cookie
LOVING	Dough—may be border line	Dough, hard sweet	Dough or hard sweet	Sponge
OPPEN	Dough or hard sweet	Dough	Sponge or dough	Sponge
SKAER	Dough	Dough	Sponge	Dough cookie
TRIEBOLD	Dough or cookie	Dough	Sponge	Sponge or dough

TABLE IV

CHEMICAL AND PHYSICAL ANALYSES OF CRACKERS [1]

Bake no.	Cracker no.	Moisture as rec'd	Protein $N \times 6.25$	Fat	Shortometer value [2]	Av. pH	Thickness of 10	Count per pound
		%	%	%			*In.*	
I	1	3.7	9.0	10.4	70.2	8.0	$2\frac{7}{8}$	140
	2	3.7	9.1	9.0	72.5	8.1	$2\frac{7}{8}$	140
	3	2.8	8.9	11.1	76.3	8.2	$2\frac{7}{8}$	140
	4	3.1	9.0	11.7	56.4	8.1	$2\frac{7}{8}$	140
II	1	2.7	9.2	8.0	67.1	8.2	$2\frac{13}{16}$	140
	2	4.3	9.1	9.4	71.3	7.9	$2\frac{13}{16}$	140
	3	3.9	9.2	7.3	62.0	8.3	3	144
	4	3.9	9.0	8.0	65.1	8.2	$2\frac{13}{16}$	140
III	1	4.9	8.75	10.7	80.0	8.2	3	138
	2	4.4	8.90	10.4	72.0	8.3	$2\frac{13}{16}$	140
	3	4.2	8.75	7.8	74.0	8.3	$3\frac{1}{16}$	142
	4	4.8	8.50	9.9	79.0	8.4	3	142
	5	4.3	8.67	9.5	70.0	8.3	$2\frac{7}{8}$	139

[1] Chemical analyses by T. E. Hollingshead.
[2] Shortometer values by H. J. Loving.

TABLE V

COMPOSITE OF SCORES AND SHORTOMETER READINGS

	Collaborator's ranking				Shortometer reading			
Cracker no.	*1*	*2*	*3*	*4*	*1*	*2*	*3*	*4*
Total	5	9	8	8	9	8	7	6
Rank	1	3	2	2	4	3	2	1

proofed, and baked under conditions similar to those reported by last year's committee.

Crackers were scored by each collaborator and the results evaluated as outlined in Cereal Chemistry **15**: 37.

Discussion of Results

The analytical data and the classifications made therefrom checked fairly well. As has been observed in the past, differences in moisture are due, no doubt, to varying conditions encountered in transit and in the laboratories before analyses are made.

Ash results indicated that flour No. 8 was lowest and flour No. 5 highest, the range in the corrected values between the two extremes being 0.04%. The range in protein was 0.7%. The flour lowest in protein was also lowest in ash, while the highest protein flour was also highest in ash.

Possibly the greatest variations occur in the reported viscosity results. Several of the committee suggested that discrepancies might

be attributed to a lack of standardization of the viscosimeters used. However, the classification of the flours from the analytical data showed splendid agreement among collaborators.

The results of the baking tests did not afford so favorable a comparison. The percentage of absorption, the loaf volume, and the characterization of all the features of the test loaves exhibited variations. It is rather surprising that, though the variations are there, the resulting classifications are in good agreement except in the case of flour No. 8. That flour was rated everywhere from a cookie flour to a sponge flour. No definite reason for such a wide variation in the classification of flour No. 8 has been advanced.

The two immediately preceding committees reported that wherever there was a difference in the rating of the flours by the two methods of testing, *i.e.*, viscosity and the baking test, the rating by the baking test was "a degree higher than by the viscosity test." The results on flours studied this year did not confirm that observation.

H. O. Triebold, in his comments on the characterizations made for flour No. 8 by the two sets of tests, has made some interesting observations which are as follows:

"Flour No. 8 gave the lowest values for ash, protein, and viscosity, after the addition of 7 c.c. of lactic acid and, on this basis, was rated as a dough or cookie flour.

"On the basis of the baking test, flour No. 8 was rated anywhere from a cookie to a cracker sponge flour. It is interesting to note that if the viscosity values on this flour be taken after the addition of 1 c.c. of lactic acid, this value is nearly that of the strongest flour, No. 7.

"A somewhat similar case may be noted in last year's report, Cereal Chemistry **15**: 35–48. There, flour No. 2 had a much higher viscosity with 1 c.c. of lactic acid added than flour No. 4, yet with 7 c.c. added both had the same viscosity. However, flour No. 2 was a stronger flour than flour No. 4 There is apparently a much closer relationship between viscosity with 1 c.c. of lactic acid added and the baking test than there is after the addition of 7 c.c. of lactic acid."

These observations indicate that a further study of this correlation might be of value. Comments by other committee members indicated preferences for certain tests, as follows:

One said, "Personally, I would rather depend upon my own classifications from the analytical tests because, being in a mill laboratory and not in a cracker plant, I have been supplying flour for specific purposes by analyses." Another writes, "I may be a bit prejudiced, however, in feeling that the chemical tests are more valuable than the baking test." A third comments, "Frankly, I do not have much confidence in the baking test alone for judging cracker flours. My

vote goes to the viscosity test as the final instrument on which to base any classification for flour for crackers or sweet goods." These comments indicate a need for further study of the possible existence of closer correlation.

The results of the cracker scores were not too encouraging. Although the performance of the flours in the shop was satisfactory, and the crackers produced met the shop requirements, a definite lack of uniformity between bakes was indicated by the collaborator's scores and by the shortometer.

Reasons for this condition are still a subject for investigation. Even when the rankings of the three bakes are composited as were those of last year's committee, there is agreement only in the ranking of crackers numbered 2 and 3. Cracker No. 3 was ranked second and cracker No. 2 was ranked third. Cracker No. 1 was rated first by the collaborators' score and fourth by the shortometer. Cracker No. 4 was ranked second by the collaborators' scores and first by the shortometer test.

With the viscosity results used as the means of classification for the flours from which the crackers were baked, the flours used in the various cracker mixes are characterized as follows:

Cracker 1 was made from the stronger sponge flour and the weaker dough flour. *Cracker 2* was made from the stronger sponge flour and the stronger dough flour. *Cracker 3* was made from the weaker sponge flour and the weaker dough flour. *Cracker 4* was made from the weaker sponge flour and the stronger dough flour.

With the baking test used as the means of classification:

Cracker 1 was made from the stronger sponge flour and a dough flour that showed variable strength. *Cracker 2* was made from the stronger sponge and the stronger dough flour. *Cracker 3* was made from a flour in the sponge rated almost unanimously as a dough flour, and a dough flour that five of the committee rated as a sponge flour. *Cracker 4* was baked from the same flour in the sponge as cracker No. 3 and the strongest dough flour.

Because of the varied characterization of flour No. 8, the fifth dough in the last series was run with that flour in the sponge and the strongest dough flour in the dough stage. The sponge was set 2° cooler than the others. While the crackers resulting from this last mix were fairly satisfactory, commercially, they were not so good as some of the others. Compared with other crackers baked in the last group the shortometer ranked cracker No. 5 first, but the collaborators ranked it fifth.

With the proper adjustment in fermentation, flour No. 8 could be used satisfactorily as a sponge flour but it would offer fewer problems

if used as a dough flour. This tends to corroborate the opinion of Reiman (1938) quoted below:

"On the basis of either the baking or viscosity tests as the measure of flour strength, the quality of the crackers seems to be more dependent upon the type of sponge flour than upon the type of dough flour used in its formula."

Conclusion and Recommendations

The work of the committee this year has been essentially a repetition of the work of the previous committee. The scope of the work is still too limited and the results not sufficiently positive to warrant definite conclusions.

However the committee does consider that added evidence is offered in support of the value of the tests used in evaluating flours for biscuit and cracker work, and that the tests merit further study.

The committee recommends:

That further study be given to the possible correlation between the classifications of flour made from the baking test, on the one hand, and the viscosity test involving the addition of 1 c.c. of lactic acid as suggested by Triebold, on the other.

That further work be done to correlate more closely the laboratory evaluation of cracker flours with their shop performance.

That some study be directed to types of cookie flour.

Acknowledgments

Appreciation of the work of the committee composed of Messrs. Armuth, Evert, Hollingshead, Knudson, Loving, Oppen, Skaer, and Triebold is gratefully acknowledged. The committee acknowledges its indebtedness to the Kroger Food Foundation for information regarding flours and sources of supply. The writer is personally indebted to Messrs. Garnatz, Putnam, Reiman, and Bayfield for their many helpful suggestions in planning the work. The crackers were baked by Perfection Biscuit Company under the supervision of Henry Hoffman and Herman Rang.

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REPORT OF THE 1937–38 COMMITTEE ON METHODS OF TESTING SOFT WHEAT [1]

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(Read at the Annual Meeting, May 1938)

During 1937–38, cereal chemists and their assistants in twenty-nine laboratories worked on projects of the Committee on Methods of Testing Soft Wheat. This committee was organized by the Executive Committee of the Association in May, 1937.

The sub-committee on Methods of Testing Cake Flour (J. W. Montzheimer, chairman) made a carefully planned collaborative study of the effects of five variations in the tentative A. A. C. C. cake-flour test. The results, printed in this issue of Cereal Chemistry, indicate that three flours can be rated in essentially the same order by each scheme of handling. Individual results, however, show the need for further study to clarify the scoring instructions to minimize the effects of the personal factor.

Since considerable dissatisfaction with the present procedure exists, and since it is not in common use, it is expected the next committee will investigate additional formulas and methods of handling.

Specifications for both loaf and layer pans were selected tentatively.

Mark A. Barmore co-operated with the sub-committee by determining in the altitude chamber at Fort Collins, Colo., the adjustments necessary for compensating the tentative formula for different air pressures from sea level to 10,000 feet.

The sub-committee on Testing Biscuit and Cracker Flours (Pearl Brown, chairman) continued the study of the usefulness of the viscosity and baking tests respectively in predicting the purpose for which a flour should be used. The practical significance of these conclusions was tested by commercial cracker bakings on the four flours used. It is expected that the succeeding committee will repeat this work on flours of known history in an attempt to eliminate variables which have crept into the work thus far, and to discover such correlations as may exist between the baking method and viscosity technique.

The sub-committee on Testing Self-Rising (and Phosphated) Flours (O. E. Gookins, chairman) undertook several individual projects for the purpose of improving the method for baking self-rising flours.

[1] General report.

These included a simplification in the method of mixing, a study of oven variables, the determination of correct water absorption, a further definition and evaluation of various items on the score card, and a further study of preserved crumb-color standards. It remains for future committees to study the baking method and scoring system with flours as the variables and to determine as far as possible what steps must be taken to eliminate further the effects of personal opinion and skill in technique.

The study of pie flours should be included in the work of this committee. However, interest was lacking among those approached this past year.

While the work has been confined to those persons acknowledged in the committee reports, an invitation is extended to other interested persons who wish to work with the committee.

WHAT THE A. A. C. C. BAKING TEST MEANS TO THE BAKER [1]

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Collaborative work on standardization of the experimental baking test was so productive in the case of hard-wheat flours that the American Association of Cereal Chemists in convention at Omaha in June, 1927, proposed a committee for the purpose of study on the question of establishing a baking test for soft-wheat flours.

Preliminary questionnaires to committee members and others deemed particularly interested in research along this line brought out the fact that while many made chemical determinations on soft-wheat flours, they felt such tests were not adequate, some being in actual doubt as to just what these determinations reflected relative to the baking qualities of a flour. Most of those answering the questionnaires thought some sort of baking test necessary in evaluating soft-wheat flours. Many had adopted the bread-baking test because they were more familiar with it and because the bread-baking test was more standardized, but no one felt that satisfactory interpretation for cake work could be made from the bread test. For the most part no scoring method was applied.

Some used a cake-baking test, the greater number having selected a white-cake formula containing shortening, others a yellow cake,

[1] Report of 1937–38 sub-committee on Testing Soft-Wheat Flours.

while several used an angel-food formula. Formulas and procedures varied as well as methods of scoring. None of those using the cake-baking test had confidence in its reflection of qualities in flour for purposes other than cake.

Those using the biscuit-baking test were principally interested in biscuit flours and self-rising flours. Formulas were within a short range of variation, scoring was on more definite lines, the whole baking test seeming to be much more standardized than the cake-baking test.

It became obvious that a baking test to be used in conjunction with chemical determinations was necessary. And as the majority of opinions favored a cake-baking test the committee formulated a tentative test to determine whether or not such a test would prove useful in reflecting other factors than characteristics of flour.

From this time forward the committee on Methods of Testing Cake and Biscuit Flours (Cereal Chemistry **5**: 301–309, 1928) has conducted collaborative tests, using suggested formulas and methods, and modifying formulas and methods in an effort to secure a test employing standardized materials as far as is feasible and having the least liability to errors through variability in time, manner of introduction of ingredients, panning, baking, etc.

The preliminary tests indicated a need for study on mixing, timing, method of scoring, and size and shape of pans. Collaborators working through subcommittees on the individual problems obtained results which led to the adoption in 1931 of the score card now used in connection with the A. A. C. C. baking test for soft-wheat flours. See score card and explanation of terms, Cereal Chemistry **8**: 253, 1931.

Viscosity, specific gravity, and other phases of mixing having been studied from several angles by the several collaborators, the white-cake formula containing shortening and mixed by the single-phase method was adopted tentatively by the organization in convention in May, 1932 (Cereal Chemistry **9**: 408, 1932).

Tolerance tests as to fat, sugar, and liquid were then added, the purpose being to show the strength of the flour in its ability to carry additional quantities of any of these or combinations of these in graded steps above the amount included in the original formula which served principally as an indicator of general baking characteristics of the flour being tested as to volume, texture, grain, color, etc. These latter tolerance tests pointed to the possibility of placing in certain ranges flours adapted to heavier types of cake and lighter goods according to tolerance to sugar, tolerance to fat, tolerance to liquid, etc., and made it possible for the commercial baker to determine in his laboratory the definite baking value of a flour before trying it out on a production basis.

In order to bring the results of the A. A. C. C. cake-baking test for soft-wheat flours more closely into correlation with results of commercial baking, frozen egg white was substituted for the egg albumin initially used in the tentative formula. Tests on the value of frozen eggs and reconstituted egg albumin carried on by collaborators showed conclusively that the frozen eggs more nearly paralleled fresh eggs, and that cakes made with frozen eggs were more truly representative cakes than sample bakings made with egg albumin and, therefore, more readily translated into terms of general production.

For general routine purposes this test may be used by the baker to check on new lots of ingredients. Very often flour will vary from lot to lot. Sometimes, while this is not enough to show up in the finished product, there are times when a simple preliminary test would check this variable and make it possible to adjust procedures accordingly. Sugar granulation very often affects cake as to crust characteristics, volume, texture, and grain. Again the laboratory test is an indicator as to what would happen in production. Shortening, too, might well be tested out first in this way. While a creaming test to a definite specific gravity is an excellent procedure to follow in general work after the creaming value and baking value of a particular lot of shortening have been determined, it is advisable to make a baking test as well since not all shortenings of the same creaming value register the same baking qualities. Eggs and milk also come under the heading of new-lot routine tests for the careful production man.

In using the A. A. C. C. baking test for soft wheat flours the Hobart C-10 mixing machine with adapter and three-quart bowls is recommended. Duplicate machines are an advantage. A comparative test is made on two ingredients, or methods, at one and the same time. Care must be exercised that the machines are synchronized as to speed and distance of paddle from bowl and these checked periodically in order to secure even volume results. Creaming shortening and checking volume or specific gravity against time are a good check on the proper set of duplicate machines. An occasional straight check baking for volume results is excellent.

Temperature becomes a very important part of such tests. Temperature control especially is more necessary in a series than in individual or single bakings. Such control may be accomplished by means of simple water baths, tempering of large batches near ovens, refrigeration, etc. It is advisable to keep records of temperatures of room, ingredients, batters, and oven, as these sometimes have important bearings upon volume, grain, texture, etc.

In using duplicate mixing equipment, the baking test becomes doubly interesting and revealing as cake from each of two mixes is baked at one and the same time. There is no chance for a variable oven temperature to bring about an effect in grain which, if the two were baked separately, might well be laid to the fault of the flour. Besides this there is the advantage of the composite cake which is unsurpassed for showing slight degrees in color differences and grain and texture. The composite cake is simply one cake made up of two batters—one-half of the weight of the total cake made up of one batter placed at one end of the pan and the remaining scaling weight made up of the second batter in the opposite end of the pan. In cakes of moderate consistency this is done easily without the aid of a temporary middle division to be taken from the batter before baking, as the two batters gradually meet in the middle of the pan and form one undivided cake. With thinner batters a temporary partition is advisable. When baked, this cake will show very clearly any differences in crust between the two mixes and, upon being cut lengthwise, will show any deviation in color, grain, or texture.

Score sheets in conjunction with simply made prints of cake slices make valuable records. For general purposes it is not always advisable to score numerically since these, having so much of the personal element entering into them, are not easily translated by others, especially upon reviewing records of work previously done. Short descriptive terms on grain, texture, color, etc., in addition to volume and weight figures, are more illuminating, especially when viewed in conjunction with the prints.

These prints may be made easily by means of stencil ink such as is ordinarily used in shipping departments, applied lightly and evenly with a paint brush to the cake slices, which in stamp-like fashion are lightly pressed, inked face down, upon paper and an impression thus obtained. Duplicate and triplicate prints may be made in this way. A slice from the middle of the cake, one half-way between middle and end, and the end slice when printed give a good cross section of the cake. When such prints are made on one sheet of paper from two cakes, together with a lengthwise slice from the composite cake of a comparative test, one has a record that, so far as volume, symmetry, grain, and texture are reflected, is more revealing than numerical scores.

Such tests are valuable in detecting differences from lot to lot of general basic ingredients and in bringing out differences between grades and types of materials. Flour differences are reflected in the type of grain and texture obtained. The A. A. C. C. formula for testing soft-wheat flours will show differences even in the batter stage

between flours of varying protein contents and will register in the baked cake differences in volume, grain, and texture, which will indicate changes advisable in manufacture by varying liquid, shortening, or sugar. Together with the tolerance tests, which are carried out by increasing the percentages of sugar, shortening, and liquid to the point of breakdown, this test places a flour for use in rich mixes such as pound or other heavy types, or light layers and cups, etc. Flours which have low sugar tolerances would indicate a cookie type of goods. Differences in granulation within the same type of flour will be reflected in the tolerance test, coarser granulations as a rule having less tolerance to shortening and sugar. Likewise the extent of aging of the flour influences the tolerance to liquid, shortening, and sugar and may be gauged by the baking test in comparison with a flour of known value, the greater extent of aging within certain limits enhancing the volume and tolerances of a flour.

As an example, suppose we were considering the purchase of flours that appeared to us to have merit along with a saving in cost but with which we had had no baking experience. A baking test by the A. A. C. C. method on samples furnished by the miller would show the qualities of these flours in comparison with a flour of known value and would give us actual knowledge of the flours upon which to base our decision.

The flours in question when baked and scored according to the A. A. C. C. method might line up as follows:

Ideal Score		Known Flour or Now in Use	Flours under Consideration	
			A	*B*
A. External				
1. Symmetry	10	9.5	10.0	9.0
2. Volume	15	14.5 (771 c.c.) [1]	15.5 (792 c.c.)	19.5 (870 c.c.)
3. Crust	5	4.0 sugary	4.5 deeper colored, sugary	4.5
B. Internal				
1. Texture	30	20	25	28
2. Grain	25	22	20	18
3. Color	15	14	14	14.5
Total	100	84	89	93.5

When tested for shortening tolerance by increasing the amount of shortening in the original formula by 50% they would perhaps reflect the following:

Ideal Score		Known Flour or Now in Use	Flours under Consideration	
			A	*B*
A. External				
1. Symmetry	10	9.0	9.0	9.75
2. Volume	15	13.5 (749 c.c.) [1]	16.0 (804 c.c.)	17.5 (828 c.c.)
3. Crust	5	3.0 macarooned	4.75 crust not as sugary	4.5 very slightly crinkled
B. Internal				
1. Texture	30	26	28	30
2. Grain	25	21	22	21
3. Color	15	14.5	14.75	15
Total	100	87.0	94.5	97.75

Under the sugar-tolerance (20% increase) test these flours might rate as follows:

Ideal Score		Known Flour or Now in Use	Flours under Consideration	
			A	*B*
A. External				
1. Symmetry	10	9.5	9.5	10.0
2. Volume	15	14.5 (776 c.c.) [1]	16.0 (797 c.c.)	17.5 (827 c.c.)
3. Crust	5	3.5 more sugary	4.5 slightly browner, tenderer	4.75 tenderer, not sugary
B. Internal				
1. Texture	30	23.0 trifle furry	25	29 velvety
2. Grain	25	22	21	20
3. Color	15	14	14	14
Total	100	86.5	89	95.25

[1] Volume figure of 780 c.c. taken as representing 100% volume count of 15. For each 20-c.c. variation, one point is added or deducted according to whether the volume is larger or smaller.

By straight volume test flour *B* is a stronger flour than *A*, which though more closely approximating the *known flour* shows better general characteristics except for color.

Under the shortening-tolerance test of 50% increase, the *known flour* falls off in volume almost 3%, while *A* becomes greater by approximately 1%, and *B* falls off by nearly 5%.

Using the sugar-tolerance test of 20% increase, the volumes of the known flour and flour *A* remained normal, flour *A* showing greater tolerance by its crust, which is tenderer and slightly browner than the known flour, which produces a more sugary exterior. Flour *B* falls

off in volume under sugar stress as it does under the increased shortening (approximately 4.5%) but shows a tenderer crust, not sugary.

Texture is improved for all three flours by the increase in shortening, the known flour showing greater improvement than flour *A* and flour *B*. Grain is improved to the same extent in flours *A* and *B*, while it falls off very slightly in the case of the known flour.

Flour *B* shows slightly better color than the others in the initial test and the shortening tolerance test; however, under the sugar-tolerance test, while not inferior to the others it is not as good in color as without the increased sugar.

Flour *A* shows a very slight increase in volume when shortening is increased. Both *A* and *B* show marked improvement in texture with the increased amount of shortening, *B* having definitely more velvety texture. Color improvement is also evident in both cases, the development of color and texture being more marked than with increased sugar content.

Flour *A* gives crusts of glossy, sugary character while *B* gives crusts that are deeper in color without the hard gloss of *A* but inclined to crepiness with increased shortening, but tenderer throughout.

Flour *B* shows qualities for velvety texture but the grain is a trifle uneven. The texture of *A* is on the feathery to furry side.

Flour *A* would most probably be best in lighter-cake mixtures, layers, cups, sponge, and angel cakes. Flour *B* would show to better advantage in heavier-type cakes, such as pound and fruit.

The effect of sugar granulations may also be reflected by means of such a baking test, and production changes made accordingly.

Comparative values of different shortenings may also be gauged by this baking test, some tending to produce larger volumes, some greasiness, others waxiness, still others oiliness, any of which would be more or less indicative of advantages in special classes of baked products.

The A. A. C. C. baking test for soft-wheat flours promises to become a dependable gauge in the laboratory of the alert baker who is interested sufficiently to develop the power of observation and translate into production lines the guide posts the test reveals.

ALTITUDE VS. BAKING POWDER USING TENTATIVE A.A.C.C. CAKE FORMULA

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(Read at the Annual Meeting, May 1938)

The purpose of the experiments herein described is to find out the amounts of baking powder necessary to produce the same grain, volume, contour, and general characteristics in cakes at different altitudes.

The formula and procedure used are as published on pages 75 to 80 of Cereal Laboratory Methods, third edition, except that fresh egg albumen and a small home-type mixer were used. (Results were, however, the same as those obtained with the Hobart mixer.) All cakes were baked 45 minutes at 183° C. (362° F.) except those at the 10,000 foot level, which were baked 55 minutes in order to eliminate the soggy streak appearing beneath the crust. All tests were run on one brand of commercial cake flour, but the final results were found to check very satisfactorily when another type of commercial cake flour was used.

A series of cakes was baked at each altitude given in Table I. The ones chosen as optimum gave the maximum volume and still maintained very satisfactory texture and grain characteristics, which were similar to those obtained with the A. A. C. C. tentative formula at sea level. An example is shown in Figure 1. The lines appearing are produced by a screen placed over the samples to serve as a reference scale, each square having an area of 4 square centimeters.

The amount of cream of tartar which produced the most satisfactory cake is given in Table I and can be expressed by the equation $y = -0.03A^2 - 0.25A + 6$ where y equals the cream of tartar in grams and A the altitude in thousands of feet. The specific gravity of the

TABLE I

Altitude	Cream of tartar	Soda	Sp. gr. batter	Weight of cake	Volume of cake	Sp. gr. cake
Sea level	6.0	3.00	0.92	272.5	780	0.349
5,000 ft.	4.0	2.00	0.91	267.3	820	0.326
7,200 ft.	2.5	1.25	0.91	262.0	790	0.331
10,000 ft.	0.5	0.25	0.92	260.5	715	0.364

batter shown is that obtained just previous to the addition of the cream of tartar, and for the entire series at all altitudes it ranged from 0.89 to 0.94 g. per c.c.

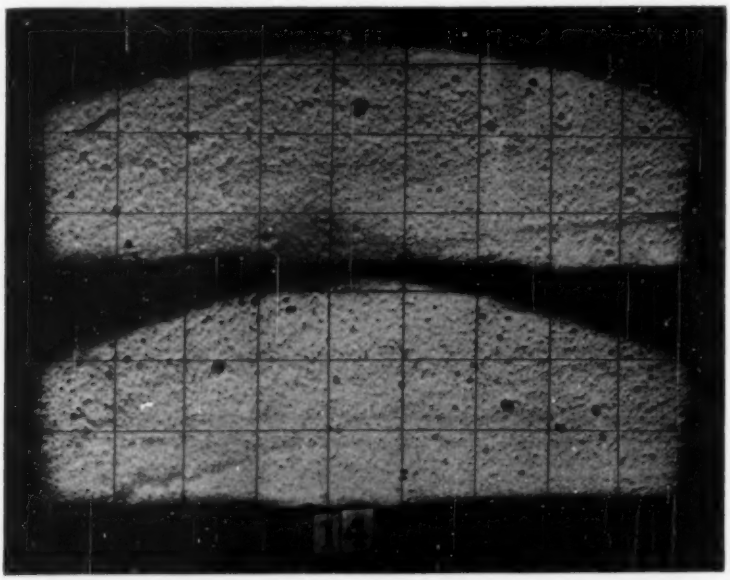

Fig. 1. Baked at sea level with the A. A. C. C. formula plus 30 g. of sugar.

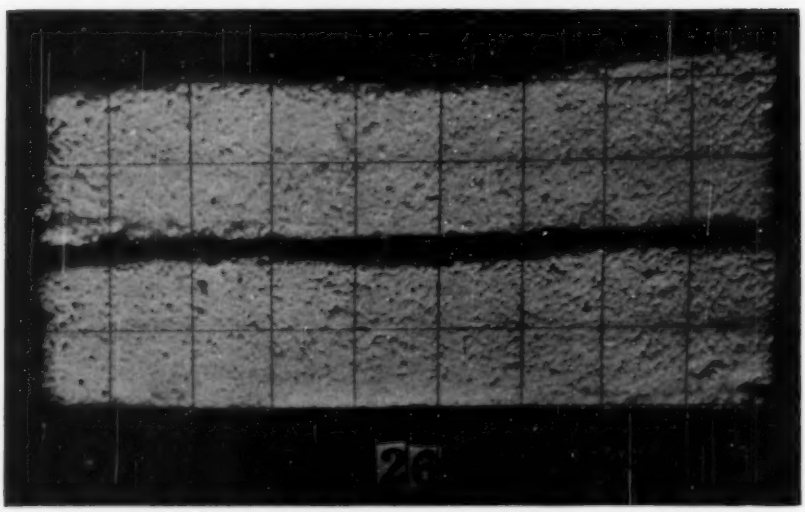

Fig. 2. Same as Figure 1 but baked at an altitude of 10,000 feet.

In order to show that the sugar tolerance is entirely different for the same flour at different altitudes, cakes were baked at sea level and at

10,000 feet using the A. A. C. C. tentative formula with an increase of 30 grams of sugar. Those baked at sea level (Fig. 1) gave excellent results, but the same cake baked at 10,000 feet fell flat (Fig. 2), although the correct amount of baking powder according to Table I was used. It would appear that at higher elevations it will be necessary to work out a chart for the sugar tolerance as well as the difference in the amount of baking powder required.

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